The Institute of Paper Chemistry

Appleton, Wisconsin

Doctor's Dissertation

The Synthesis and Acid Hydrolysis of
Methyl Alpha-D-Glucopyranosiduronic Acid

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June, 1961

THE SYNTHESIS AND ACID HYDROLYSIS OF METHYL ALPHA-D-GLUCOPYRANOSIDURONIC ACID

A thesis submitted by

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of The Institute of Paper Chemistry
for the degree of Doctor of Philosophy
from Lawrence College,
Appleton, Wisconsin

June, 1961

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INTRODUCTION

THE OCCURRENCE OF ALDOBIOURONIC ACIDS

An aldobiouronic acid may be defined as a disaccharide in which one of the sugar components is a uronic acid linked in glycosidic union to a hexose or pentose sugar (1). The aldobiouronic acid most commonly obtained from wood is 2-O-(4-O-methyl-α-D-glucopyranosyluronic acid)-D-xylose, shown in Fig. 1.

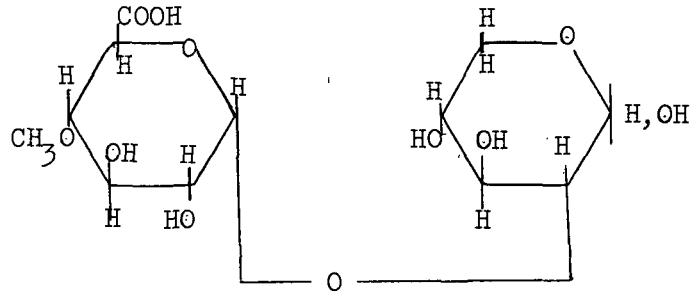

Figure 1. 2-O-(4-O-Methyl-α-D-Glucopyranosyluronic Acid)-D-Xylose

In the hemicellulose fraction of wood the aldobiouronic acid is thought to exist as a part of the polymer, 4-O-methyl glucuronoxylan, shown in Fig. 2 (2). Xylose and the aldobiouronic acid are the main products ordinarily obtained upon acid hydrolysis of this polymer. It is the strong resistance of the glycosidic bond of the aldobiouronic acids that has led to their ready isolation. This unusual stability of the biouronic linkage has prompted some speculation and a small amount of research.

Common names of sugars will be used throughout this discussion. They will be introduced like the following example: methyl α-D-glucopyranosiduronic acid (methyl α-glucuronide).

Before published work on this problem is discussed, however, the fundamentals of glycoside hydrolysis will be reviewed.

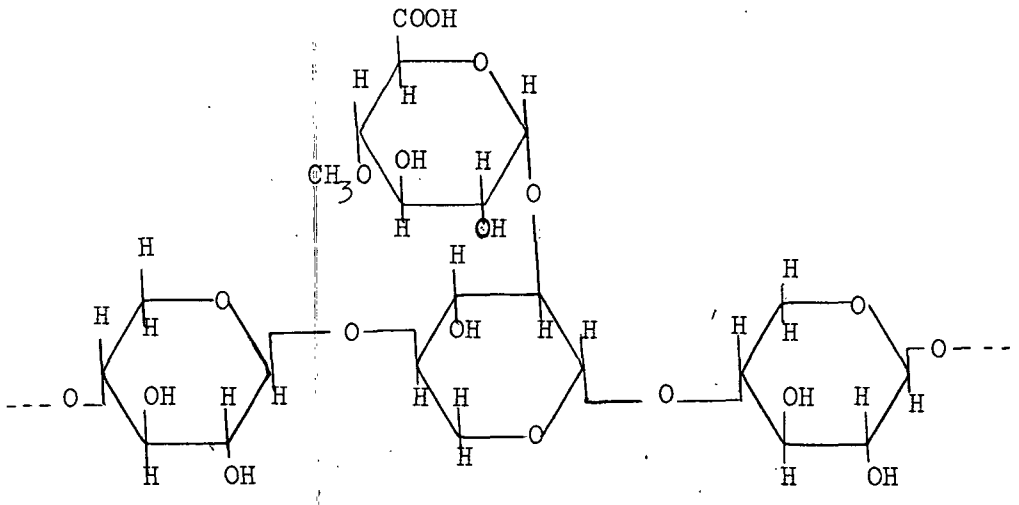

Figure 2. 4-O-Methyl Glucuronoxylan

ACID HYDROLYSIS OF GLYCOSIDES

FACTORS THAT INFLUENCE THE RATE OF GLYCOSIDE HYDROLYSIS

Because an extensive discussion of this topic has been presented by Shafizadeh (3), only the main points are reviewed here.

Steric Strain and Constitution of the Sugar

Five- and seven-membered rings are strained by the distortion of the tetrahedral valency angle and, therefore, have a higher free energy than pyranose rings. If it is assumed that all three types of ring compounds are hydrolyzed through the same intermediate or through intermediates at the same energy level, it is evident that the energy of activation for pyranosides should be greater than for the other two ring forms. This

difference in activation energy should explain the generally faster hydrolysis of furanosides.

The amount of strain in the well-staggered pyranose rings originates from the nonbonded interaction of axial groups and other conformational instability factors. Thus, steric strain can account for some of the differences in rate of hydrolysis between the various pyranosides.

Nature of the Aglycon

The aglycon group is the aryl, alkyl, or sugar group linked through the glycosidic bond to the glycosyl group. The glycosyl moiety is that sugar radical whose reducing group enters into formation of the glycosidic bond.

The role of the aglycon in glycoside hydrolysis is not well understood. However, it has been generally found that glycosides having large aglycons, e.g., sugars, are more readily hydrolyzed than those with small aglycons, e.g., methanol.

Substitution of the Glycosides

Shafizadeh has divided substitution influence into masking of the reactive center, modifications of the strain and conformational stability of the molecule, and polar effects of the substituents.

Masking of the reactive center is considered to be the reason for the stability of methyl 2-amino-2-deoxy-D-glucopyranoside. The positively charged amino group is thought to decrease the concentration of hydronium ions in the vicinity of the glycosidic group.

Fully hydroxylated glycosides are generally more resistant to acid hydrolysis than are deoxy glycosides. The differences in behavior of these two classes of glycosides have been attributed to both polar effects and modifications of the strain and conformational stability of the molecules. The exact means by which the polar effects, strain modifications, etc., promote or retard hydrolysis are controversial.

POSTULATED REACTION MECHANISM

The reaction mechanism for the hydrolysis of methyl and phenyl α- and β-D-glucopyranosides has been studied by Bunton, et al. (4). They proved that only hexose-oxygen bonds were broken in hydrolysis. Their experimental work yielded a linear relationship between the log of the first-order rate constant and the Hammett acidity function, H_o. Their conclusion was that the hydrolysis of methyl and phenyl glucopyranosides proceeds through the unimolecular decomposition of the glycoside's conjugate acid (protonated glycoside). This is analogous to the well-established mechanism for the acid hydrolysis of acetals (5). The Hammett acidity function, qualitatively, expresses the tendency of a solution to transfer a proton to a neutral base (6-8).

The theoretical work of Bunton, et al. has been illustrated in the following way: $\underline{G}$ and $\underline{G}^+\underline{H}$ represent the glycoside and conjugate acid respectively, and $\underline{G}_1^+$ represents some other entity formed from $\underline{G}^+\underline{H}$.

$$\underline{G} + H_3O^+ \rightleftharpoons \underline{G}^+\underline{H} + H_2O \qquad \text{fast}$$

(a)
$$\underline{G}^+\underline{H} \longrightarrow \underline{G}_1^+ \qquad \text{slow}$$
$$\underline{G}_1^+ + H_2O \longrightarrow \text{products} \qquad \text{fast}$$

(b)
$$\underline{G}^+\underline{H} + H_2O \longrightarrow \text{products} \qquad \text{slow}$$

They proved that since the rate-controlling step of the reaction was unimolecular, (a) was the correct scheme rather than the bimolecular (b).

Two possible pathways for the unimolecular hydrolysis of glycosides have been proposed by Bunton, et al. There was no simple way of choosing between them. These pathways are presented in detail by Shafizadeh (3, 9) and in a somewhat simplified form in Fig. 3.

Novikova and Konkin (10) have found evidence that a similar mechanism is followed in the homogeneous hydrolysis of cellulose, cellobiose, and xylan.

HYDROLYSIS OF URONOSIDES

Uronosides are glycosides of uronic acids. Therefore, aldobiouronic acids belong to this category.

The resistance to hydrolysis of the common wood aldobiouronic acid has been quantitatively demonstrated by Whistler and Richards (11). They prepared the neutral disaccharide, 2-O-(4-O-methyl-α-D-glucopyranosyl)-D-xylitol, by reduction of the aldobiouronic acid. This neutral disaccharide was found to undergo acid hydrolysis at about the same rate as maltose and 20 times the rate of the aldobiouronic acid. Whistler and Richards attributed the hydrolysis resistance of the aldobiouronic acid to the presence of the carboxyl group, and they felt that the carboxyl group stabilized the glycosidic bond primarily by means of an inductive effect.

Marchessault and Rånby (12) discussed the inductive effect of the carboxyl group in terms of Bunton's (4) theory of glycoside hydrolysis.

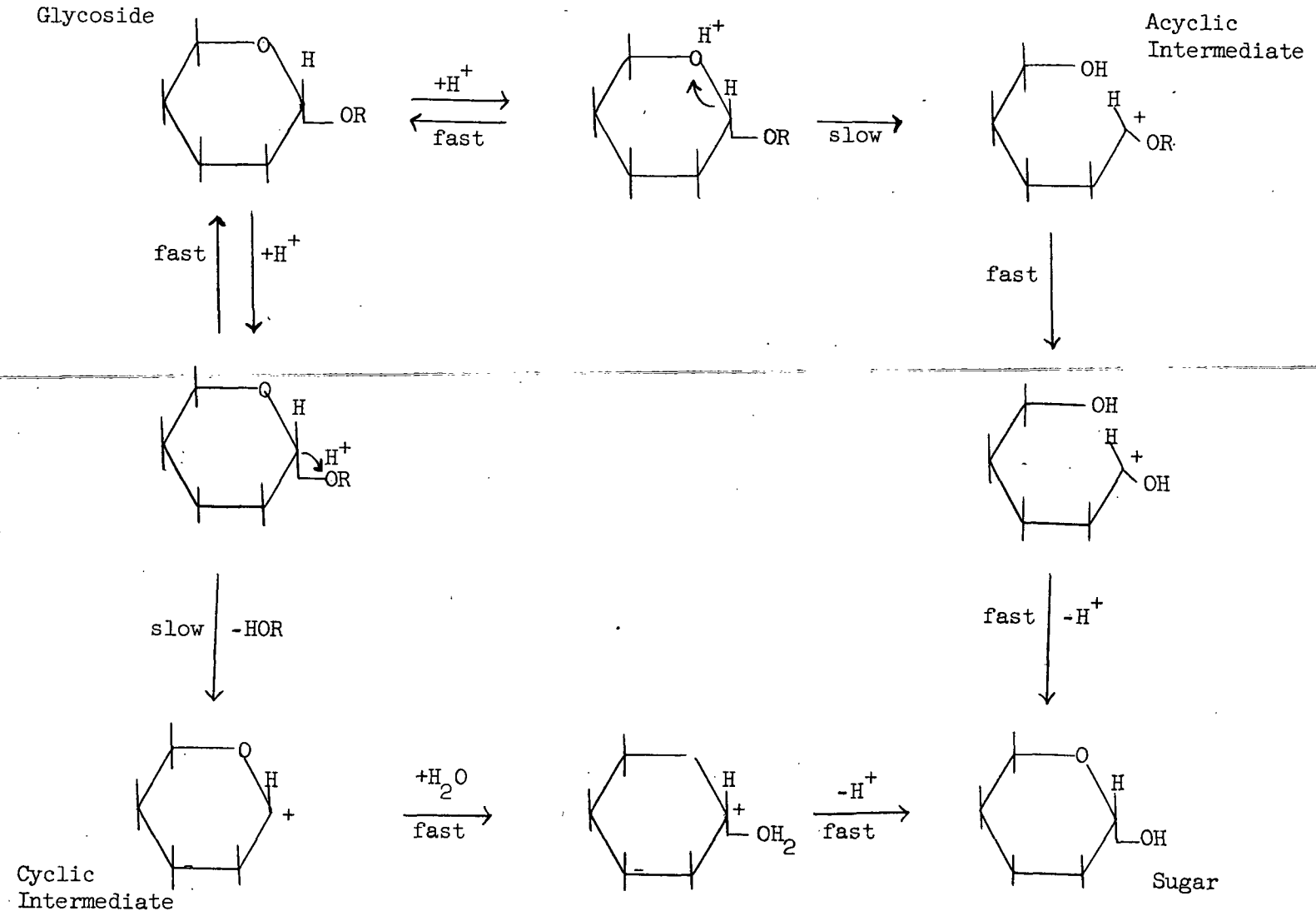

Figure 3. Proposed Pathways for Glycoside Hydrolysis

Their discussion necessarily included the tacit assumption that the cyclic pathway of the Bunton mechanism applied to uronoside hydrolysis.

Hamilton and Thompson (13) have also attributed the stability of aldobiouronic acids to the electron-attracting ability of the carboxyl group. However, the exact means by which the carboxyl group stabilizes the glycosidic bond, according to their theory, is somewhat similar to the interpretation of Marchessault and Rånby.

Until quite recently, the only other important work on the hydrolysis of uronosides was that conducted by Morell and Link (14). They compared rates of acid hydrolysis of methyl α-D-galactopyranoside (methyl α-galactoside) and methyl α-D-galactopyranosiduronic acid (methyl α-galacturonide). Under the hydrolysis conditions employed they found very similar first-order rate constants for the two compounds. Moreover, experimental activation energies found were 29 kilocalories per mole for methyl α-galacturonide and 35 kilocalories per mole for methyl α-galactoside. The difference in rates of hydrolysis of these oxidized and unoxidized simple glycosides contrasts sharply with that difference in rates of hydrolysis of oxidized and unoxidized disaccharides discussed by Whistler and Richards (11).

Very recently, Nakano and Rånby (15) have presented data on the dilute acid hydrolysis of methyl α-D-glucopyranoside (methyl α-glucoside) and potassium (methyl α-D-glucopyranosid)uronate (potassium salt of methyl α-glucuronide). Methyl α-glucoside was hydrolyzed at about twice the rate of the potassium salt of methyl α-glucuronide. This difference in rates

was again attributed to an inductive effect involving the carboxyl group. It is noteworthy that the twofold difference in rates found here and the twentyfold difference found by Whistler and Richards (11) have been attributed to the same cause.

ACID DEGRADATION OF URONIC MATERIALS

Whenever uronic materials are treated with acid, some degradation occurs (16). Products that usually result from this degradation are carbon dioxide, furfural, and reductic acid (1). Stutz and Deuel (17) have proposed a mechanism for the degradation of uronic acids. It is similar to mechanisms proposed for the degradation of pentoses (18, 19) in that they all involve successive dehydration steps. Another very significant point of similarity of these proposed mechanisms is that the aldehyde group of the compound degraded is involved in the initial step of each mechanism.

Studies of decarboxylation have further emphasized the importance of the carbonyl group. Uronic acids yield approximately theoretical quantities of carbon dioxide. Vigorous treatments over long periods of time have been required to obtain even small amounts of carbon dioxide from such nonuronic, nonreducing compounds as glucaric acid, gluconic acid, and oxalic acid (20). By way of contrast, keto aldonic acids have been found to yield carbon dioxide readily (1).

Gould (21) has pointed out that "almost any carboxylic acid . . . can be made to suffer decarboxylation if it is treated severely enough . . . ." He further pointed out that decarboxylation occurs more readily

if a point of unsaturation or an electrophilic group like a carbonyl group is present in the carboxylic acid. Therefore, it appears probable that the carbonyl group is responsible for the decarboxylation of uronic acids and keto aldonic acids. Stutz and Deuel (17) have also presented this view. The small amounts of carbon dioxide that Taylor, et al. (20) obtained from the nonuronic acids probably were liberated because the compounds were "treated severely enough."

From a study of decarboxylation of pectins Conrad (22) concluded that hydrolysis precedes decarboxylation. On the basis of the discussion given by Gould (21), this would appear quite reasonable. If decarboxylation did occur before hydrolysis, it would probably have to be initiated by protonation of a secondary hydroxyl group. But according to Edward (23), "The basic strength of the secondary alcohol group . . . is little greater than that of water and the extent of its protonation in dilute acid will be extremely slight."

CHOICE OF MATERIAL FOR STUDY

A significant contribution to the chemistry of uronosides might result from a fundamental study of the acid hydrolysis and degradation of the aldobiouronic acid commonly obtained from the partial hydrolysis of wood. It may be hypothesized that following hydrolysis of the glycosidic bond, carbon dioxide would be evolved from the glucuronic acid moiety, and that probably furfural and reductic acid would be formed from both the glucuronic acid and xylose portions of the original molecule. Though such an investigation would be valuable, the system appears to be very complex.

The contrast between the results of Whistler and Richards (11) and Morell and Link (14) (page 7) raises interesting questions about the generality of uronoside stability. Is the extraordinary stability of the aldobiouronic acid toward dilute acid hydrolysis exhibited by other glycosides of glucuronic acid where the hydrogen on the hemiacetal hydroxyl of the glucuronic acid is replaced by an organic radical? Does methyl α-galacturonide's normal stability extend to other uronosides possessing a methyl aglycon? A study of the acid hydrolysis and degradation of methyl α-D-glucopyranosiduronic acid (methyl α-glucuronide) (Fig. 4) was chosen to help to answer these questions.

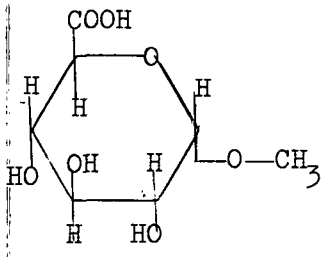

Figure 4. Methyl α-D-Glucopyranosiduronic Acid

From the structure of methyl α-glucuronide, it was expected that hydrolysis and degradation should result in a much less complicated system than that foreseen for the breakdown of the aldobiouronic acid.

STATEMENT OF THE PROBLEM

In comparison with 2-O-(4-O-methyl α-D-glucopyranosyl)-D-xylitol, 2-O-(4-O-methyl-α-D-glucopyranosyluronic acid)-D-xylose was found to be quite resistant to acid hydrolysis (11). Though the reasons given for the stability of the above aldobiouronic acid should be expected to apply to all uronosides, methyl α-galacturonide was found not to be similarly resistant. These results suggested that further understanding of uronoside hydrolysis was needed.

Methyl α-glucuronide was considered an appropriate uronoside for further study; it has a glycosyl group similar to that of the aldobiouronic acid and an aglycon identical to that of methyl α-galacturonide.

Therefore, the purpose of this investigation was to determine the conditions necessary for acid hydrolysis of methyl α-glucuronide and the extent of concomitant degradation. It was desired to obtain from these results an indication of the generality of resistance to hydrolysis of uronosides. [It should be pointed out that this study had been planned and nearly completed at the time the results of Nakano and Rånby (15) (page 7) were presented.]

This investigation was divided into two parts: (a) a study of the synthesis of methyl α-glucuronide of high purity, and (b) a study of the acid hydrolysis and degradation of methyl α-glucuronide.

RESULTS AND DISCUSSION

SYNTHESIS OF METHYL α-GLUCURONIDE*

CATALYTIC AIR OXIDATION

The catalytic air or oxygen oxidation of methyl α-glucoside was first used for the preparation of methyl α-glucuronide by Mehltretter (24). This oxidation was also used by Barker, Bourne, and Stacey (25), who implied that the reaction was quite specific. A characteristic of these syntheses is that the methyl α-glucuronide was not isolated in a pure form. The synthesis of methyl α-glucuronide has been further discussed in a review by Mehltretter (24a).

Methyl α-glucoside was oxidized by the method of Mehltretter, et al. (26). As the oxidation proceeded, sodium bicarbonate was added to neutralize the acid formed. When the reaction reached 80% completion, as determined by the amount of bicarbonate added, it was stopped; and the reaction mixture was filtered. Sodium ions were removed on a column of cation-exchange resin; then the solution was neutralized with barium hydroxide. The solution was placed in the refrigerator for several days during which time insoluble barium salts of impurities precipitated.

Barium ions were removed from small amounts of the above material, and samples of the solution and the precipitate were chromatographed.

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*Detailed procedures for this synthesis are presented in Appendix I, p. 50.

The material that had formed the insoluble salts appeared to consist of a large number of compounds. The material which remained in solution exhibited two spots, the larger of which was shown later to be methyl α-glucuronide. On a separate chromatogram the smaller of the two spots gave a light orange color when sprayed with 2,4-dinitrophenylhydrazine. A subsequent spray with alcoholic sodium hydroxide turned the spot blue. This indicated that the smaller spot might have been a carbonyl-containing compound, but it was studied no further.

After precipitation of the impurities was allowed to occur for several days, the oxidation mixture was filtered, concentrated, and poured into absolute ethanol. This precipitated the crude barium salt of methyl α-glucuronide as a white floc and left the unreacted methyl α-glucoside in solution. Calculated methoxyl and barium analyses for possible forms of methyl glucuronide are compared with data from the isolated crude product in Table I.

TABLE I

ANALYSIS OF
BARIUM (METHYL α-D-GLUCOPYRANOSID)URONATE

		Methoxyl, %	Barium, %
Calculated--	Anhydrous	11.22	24.90
	Monohydrate	10.88	24.10
	Dihydrate	10.54	23.38
Found--	Crude product	10.8	26.5
	Purified product	11.00	23.96

The chromatograms and these analyses support the conclusion that the catalytic oxidation was not specific.

HYDRAZIDE PREPARATION

The barium was removed from an aqueous solution of the crude product. Concentration of the aqueous solution followed by repeated concentration from large quantities of absolute ethanol yielded a dry sirup of crude methyl α-glucuronide.

Results of preliminary work in which a separate sample of uronoside was esterified by refluxing with methanolic hydrogen chloride suggested that this treatment caused anomerization. To avoid this, the uronoside was dissolved in methanol and esterified with a large excess of ethereal diazomethane. Titration of a small sample of the ester showed that only 0.4% free acid remained.

The ester solution was concentrated and then reacted with hydrazine according to the method of Wolfrom, Kowkabany, and Binkley (27). Crystallization occurred within 15 minutes. Repeated recrystallization from water-ethanol produced a product melting at 231.5-232.5°C. with a specific optical rotation of +150° (c, 0.623 in water). Hardegger and Spitz (28) reported the following constants for methyl α-glucuronide hydrazide (Fig. 5) prepared in a completely different way: m. 234°C., $[\alpha]_D$ +151° (c, 1.0 in water).

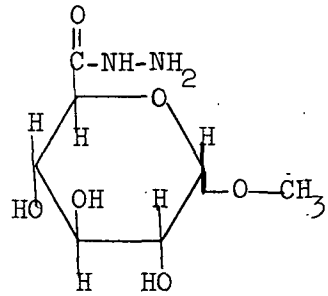

Figure 5. Methyl α-Glucuronide Hydrazide

The isolation of this hydrazide represents the first proof by means of a crystalline derivative that methyl α-glucuronide was a product of the catalytic air oxidation.

HYDROLYSIS OF THE HYDRAZIDE WITH BARIUM HYDROXIDE

Several methods have been published for the hydrolysis of hydrazides or phenylhydrazides of aldonic acids. The method of Hann and Hudson (30) utilized boiling copper sulfate, and that of Thompson and Wolfrom (31) used nitrous acid. These methods were judged unsuitable for use in splitting the hydrazide of a uronoside, because of the high probability of their attacking the glycosidic bond.

Fischer and Passmore (32) devised a method for splitting aldonic phenylhydrazides with barium hydroxide, but Hann and Hudson (30) later found it unsatisfactory for that purpose. Nevertheless, a modified Fischer and Passmore method was used in the present investigation.

The hydrazide was refluxed for 10 minutes in 5% barium hydroxide solution. Excess barium was removed, and the product was isolated as its barium salt by precipitation in absolute ethanol.

After removal of the barium ions from a small sample of the product, chromatograms indicated: (a) No unreacted hydrazide remained. (b) The spot of this purified product corresponded to the spot of the main component of the crude oxidation reaction mixture. (c) The purified product was chromatographically homogeneous.

Any occluded alcohol was removed by drying a small sample of an aqueous sirup of the purified product in an Abderhalden drier. Analysis of this purified product showed: methoxyl, 11.00%; barium, 23.96%. These data are within 1% of the calculated values for the monohydrate barium salt of methyl glucuronide, as seen in Table I (page 13). Hardegger and Spitz (29) have reported the barium salt of methyl β-glucuronide to be a monohydrate.

The specific optical rotation of the barium salt of methyl α-glucuronide was found to be +118° (c, 0.577 in water). After a quantitative removal of barium from a sample of the salt, the specific optical rotation of the resulting free acid was +167° (c, 0.133 in water).

The synthesis described here is the first synthesis of the barium salt of methyl α-glucuronide having a high degree of purity. This also is the first demonstration of the lability to basic hydrolysis of the hydrazine portion of methyl α-glucuronide hydrazide.

This synthesis was repeated several times and finally produced about 90 grams of recrystallized hydrazide. The hydrazide was split in batches as the barium salt or free acid methyl α-glucuronide were needed for subsequent study.

Nakano and Rånby's (15) synthesis of the potassium salt of methyl α-glucuronide also utilized the catalytic oxidation of methyl α-glucoside. They used a chromatographic purification. The desired spot of their potassium salt was eluted from a heavy paper chromatogram, dried, and characterized by methoxyl determination. After removal of cations, the product of Nakano and Rånby and the product of this study appeared to be chromatographically identical. The chromatographic sample of Nakano and Rånby's preparation was provided through the courtesy of Dr. Rånby.

ACID HYDROLYSIS AND DEGRADATION OF METHYL α-GLUCURONIDE*

HYDROLYSIS OF METHYL α-GLUCURONIDE AND METHYL α-GLUCOSIDE

Preliminary Considerations

It was decided that results would be most valuable if the initial portion of the hydrolysis, i.e., less than 5% hydrolysis, were studied. In this range, side reactions would be minimized.

The use of methyl glucuronide and glucuronic acid determinations to follow the hydrolysis was considered, but experimental difficulties were foreseen in attempting to determine small amounts of glucuronic acid in the presence of large amounts of methyl α-glucuronide.

Determination of methanol was the means chosen for following cleavage of the glycosidic bond. Methanol should not be degraded in the acidic

*Detailed procedures for this study are presented in Appendix I, p. 53.

system, and small amounts of methanol can be readily determined. The specific nature of methanol determinations contrast with the somewhat abstract nature of the optical rotation measurements used by Nakano and Rånby (15). In relating optical rotation changes to uronoside hydrolysis, Nakano and Rånby made the tacit assumption that the amounts of glucuronic acid degradation occurring were insignificant. It has long been known that uronic acids are degraded in mineral acid systems, but prior to the present study there was no information on the extent of this degradation in the type of system studied by Nakano and Rånby.

Hydrolysis Studies

Barium ions were removed from a sample of the barium salt of methyl α-glucuronide. A 0.241 molar solution of methyl α-glucuronide in normal sulfuric acid was prepared. Hydrolyses were conducted in 2-ml. glass ampoules which were immersed in a constant temperature bath and removed at various times. After hydrolysis, the methanol was distilled from the neutralized contents of each ampoule and determined by the chromotropic acid method of Boos (33). Studies were conducted at 70, 80, and 90 ± 0.1°C.

An identical procedure was followed in hydrolyzing methyl α-glucoside, except that the glycoside concentration was 0.257 molar. Methyl α-glucoside was studied for comparative purposes; however, to the author's knowledge, its hydrolysis has never before been studied by means of methanol determinations.

Results and Comparisons

Results from the acid hydrolysis of methyl α-glucuronide and methyl α-glucoside are shown in Fig. 6-12. The displacement from the origin of the intercept of some of the plots is the result of plotting time data not corrected for heat-up of the ampoule contents.

Pseudo first-order rate constants are shown in Tables II and III.

TABLE II

FIRST-ORDER RATE CONSTANTS FOR METHYL α-GLUCURONIDE HYDROLYSIS

Temperature, °C.	First Order Rate Constant, $min.^{-1} \times 10^3$	Correlation Coefficient
70 ± 0.1	0.113 ± 0.0088	-0.998
80 ± 0.1	0.413 ± 0.017	-0.999
90 ± 0.1	1.45 ± 0.16	-0.994

TABLE III

FIRST-ORDER RATE CONSTANTS FOR METHYL α-GLUCOSIDE HYDROLYSIS

Temperature, °C.	First-Order Rate Constant, $min.^{-1} \times 10^3$	Correlation Coefficient
70 ± 0.1	0.177 ± 0.0014	-0.999
80 ± 0.1	0.748 ± 0.11	-0.994
90 ± 0.1	3.14 ± 0.21	-0.997

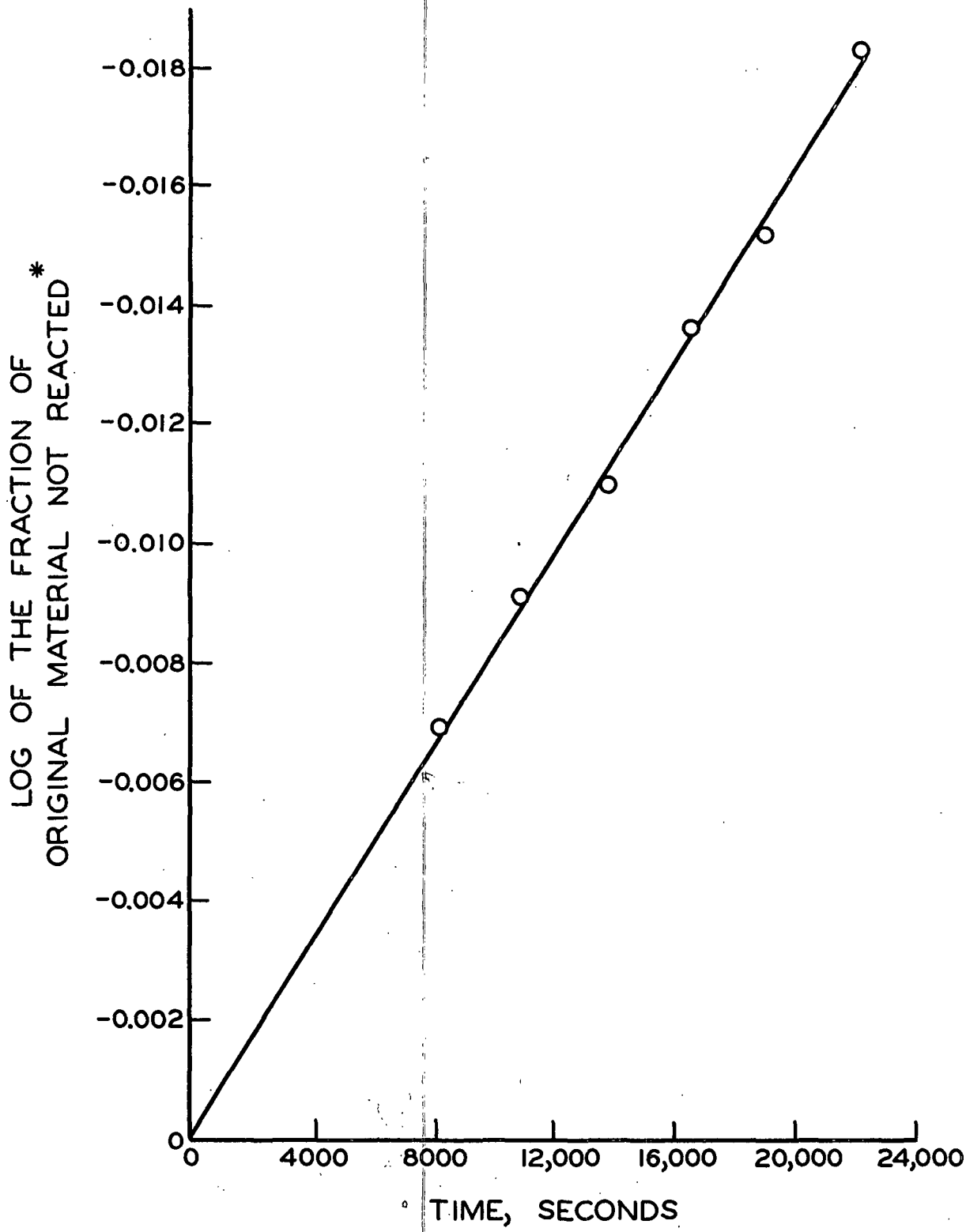

Figure 6. Hydrolysis of Methyl α-Glucuronide at 70 ± 0.1°C.

* Calculated from methanol evolution data as described in Appendix II. The methanol found is considered to be equivalent to the amount of methyl α-glucuronide hydrolyzed.

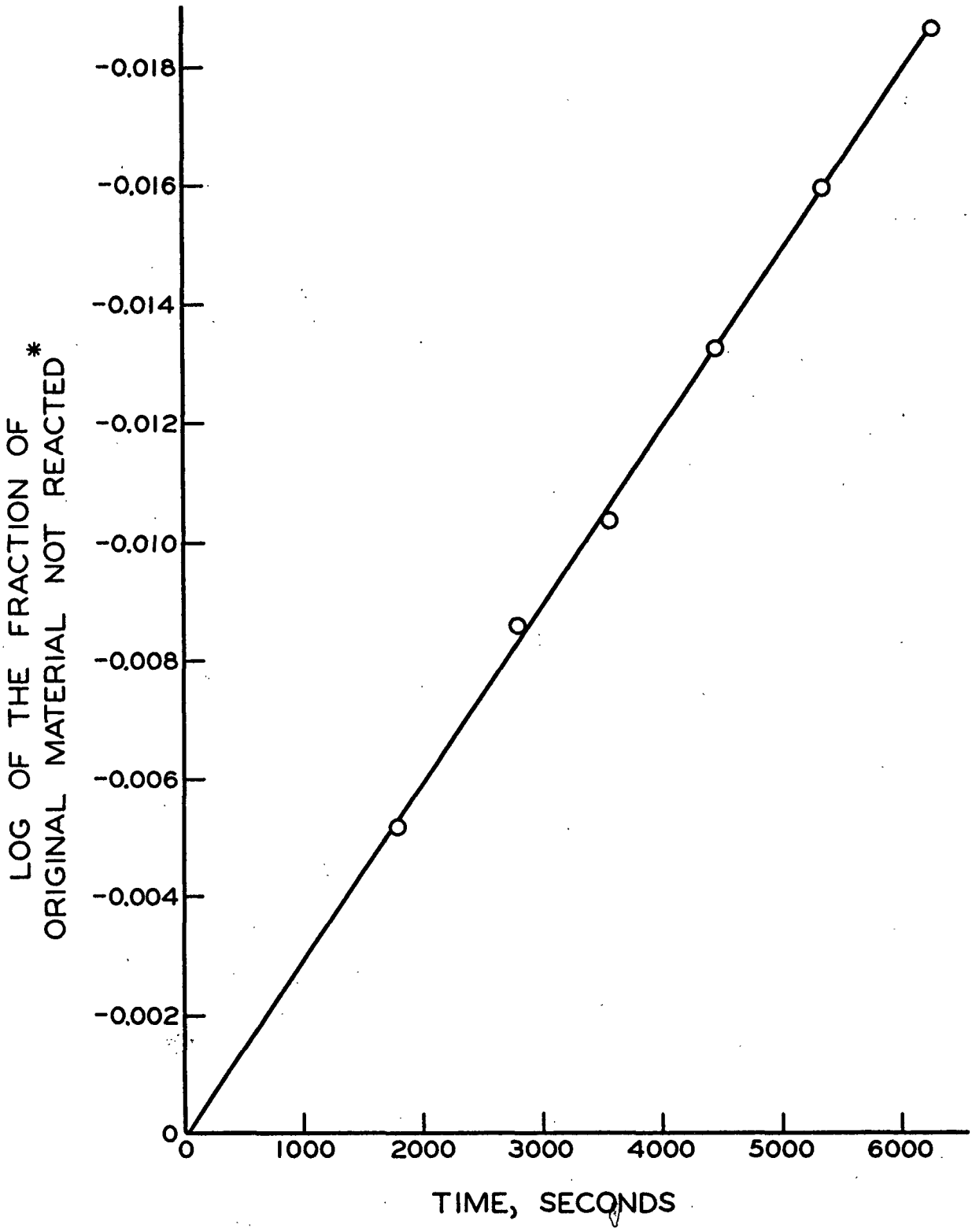

Figure 7. Hydrolysis of Methyl α-Glucuronide at 80 ± 0.1°C.

* Calculated from methanol evolution data as described in Appendix II. The methanol found is considered to be equivalent to the amount of methyl α-glucuronide hydrolyzed.

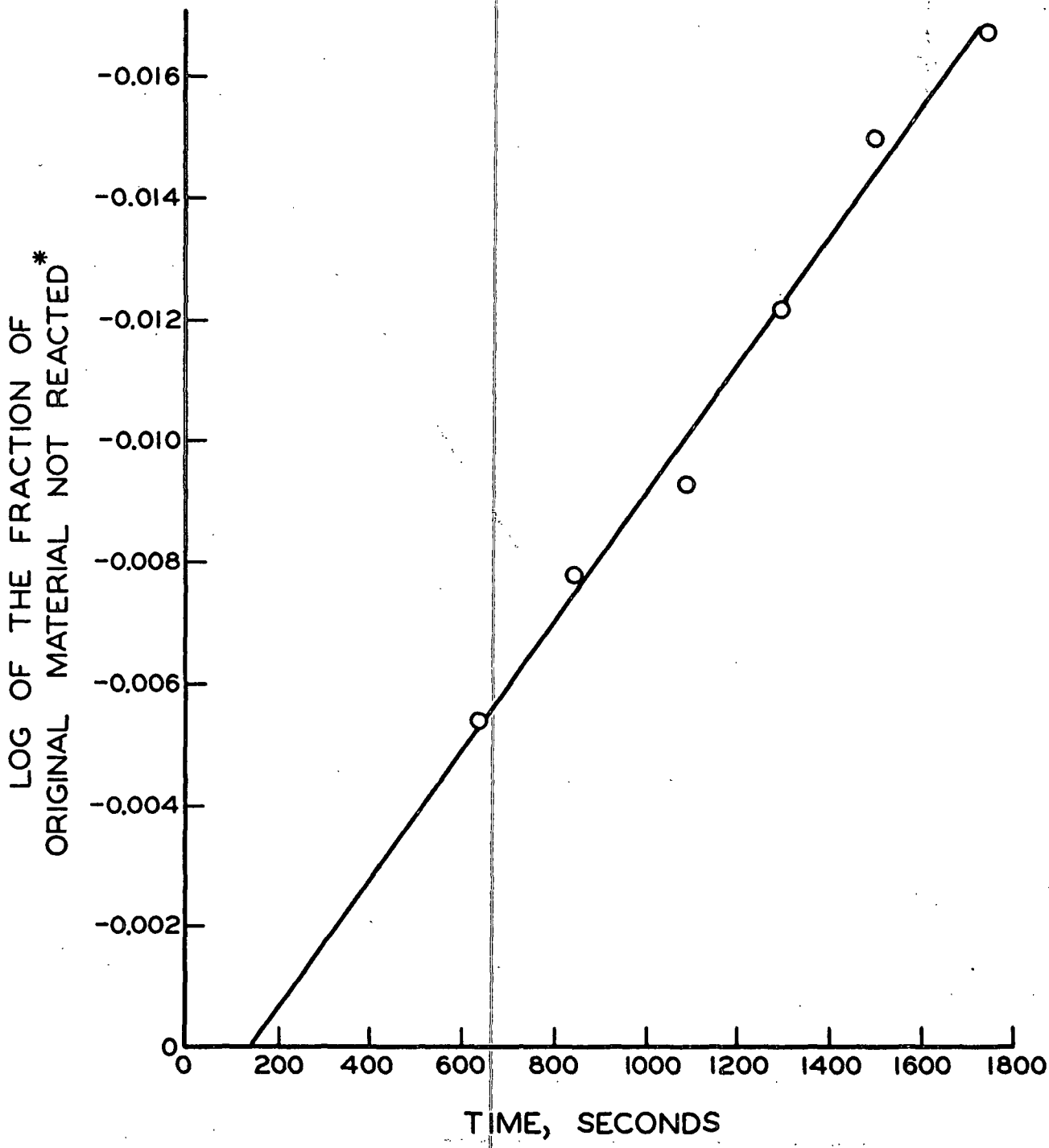

Figure 8. Hydrolysis of Methyl α-Glucuronide at 90 ± 0.1°C.

* Calculated from methanol evolution data as described in Appendix II. The methanol found is considered to be equivalent to the amount of methyl α-glucuronide hydrolyzed.

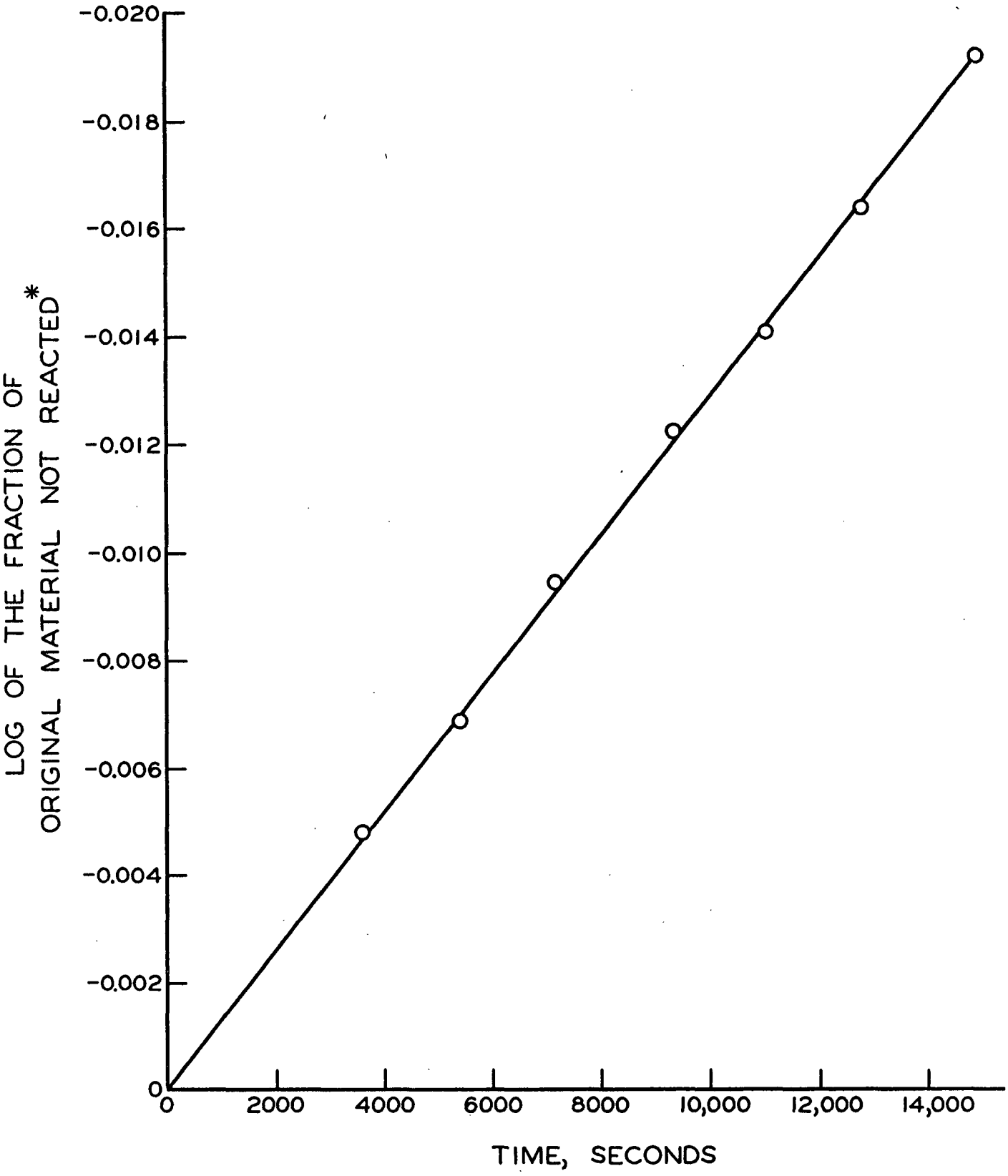

Figure 9. Hydrolysis of Methyl α-Glucoside at 70 ± 0.1°C.

* Calculated from methanol evolution data as described in Appendix II. The methanol found is considered to be equivalent to the amount of methyl α-glucoside hydrolyzed.

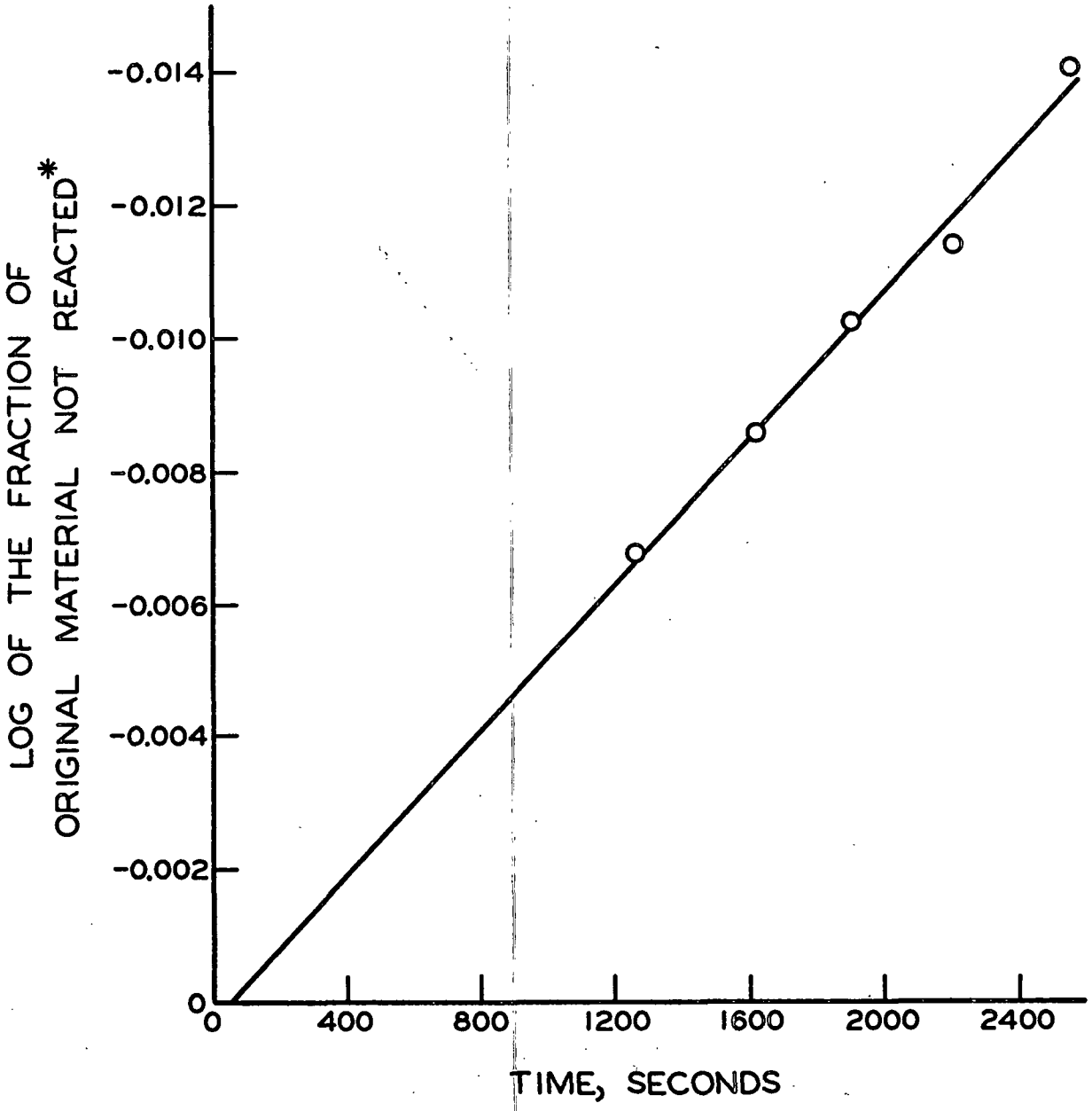

Figure 10. Hydrolysis of Methyl α-Glucoside at 80 ± 0.1°C.

* Calculated from methanol evolution data as described in Appendix II. The methanol found is considered to be equivalent to the amount of methyl α-glucoside hydrolyzed.

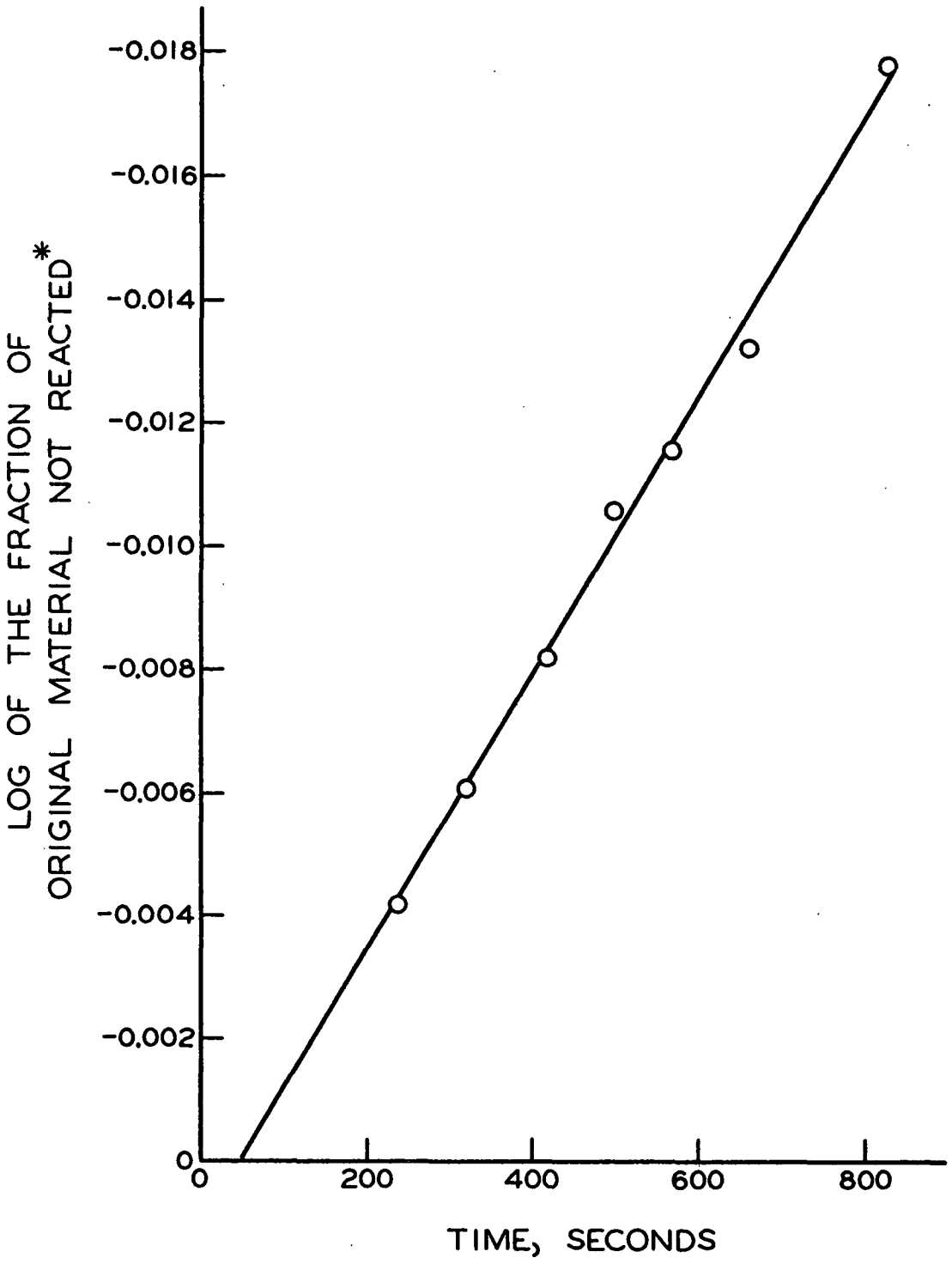

Figure 11. Hydrolysis of Methyl α-Glucoside at 90 ± 0.1°C.

* Calculated from methanol evolution data as described in Appendix II. The methanol found is considered to be equivalent to the amount of methyl α-glucoside hydrolyzed.

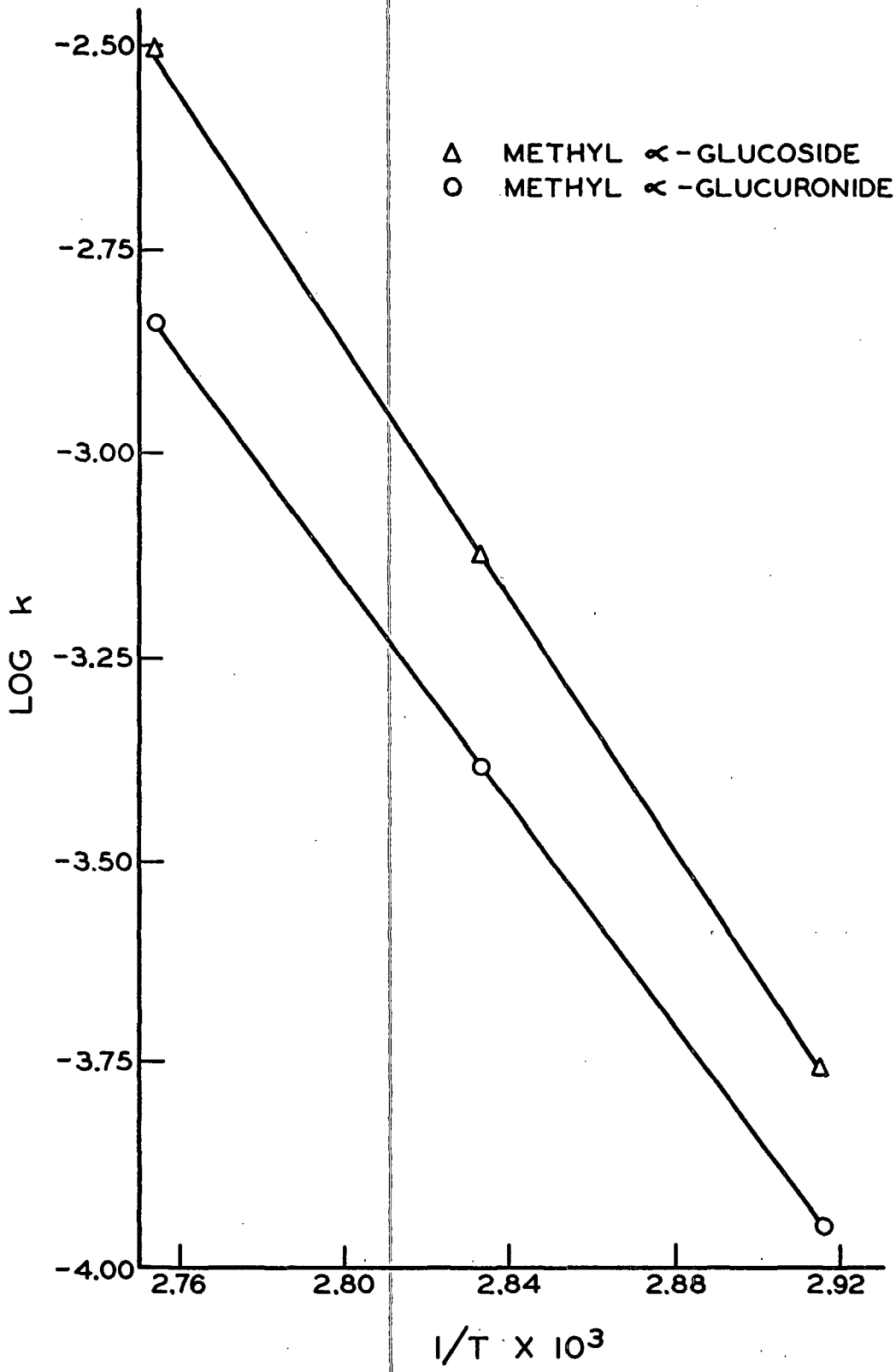

Figure 12. Log First Order Rate Constant vs. Reciprocal Absolute Temperature

The linear relationship between the logs of the rate constants and reciprocal absolute temperature, shown in Fig. 12, enabled energies of activation to be calculated. Both of these lines have correlation coefficients of -0.999. The activation energy for methyl α-glucuronide hydrolysis, 31,600 ± 2,370 calories per mole (90% confidence limits), is insignificantly different from that which was calculated from Nakano and Rånby's (15) data, 30,000 calories per mole.

Available activation energies for methyl α-glucoside hydrolysis are shown in Table IV. The extreme values in Table IV, those from the data of Nakano and Rånby and Moelwyn-Hughes, are not firmly established, because they were computed from slopes of lines drawn between only two data points. Therefore, the good agreement between results of this study and those of Heidt and Purves fixes within narrow limits the activation energy for methyl α-glucoside hydrolysis at approximately 35,000 calories per mole.

TABLE IV

REPORTED ACTIVATION ENERGIES
FOR METHYL α-GLUCOSIDE HYDROLYSIS

Source	Activation Energy, calories per mole
Nakano and Rånby (15)	31,000
Heidt and Purves (34)	34,780 ± 360
This investigation	35,600 ± 927
Moelwyn-Hughes (35)	38,190

The first-order rate constants in Tables II and III have been found to be from two to three times the magnitude of similar constants interpolated from the data of Nakano and Rånby (15). Because theoretical reasons for these differences are not evident, the following comparisons have been made in order to clarify the differences and evaluate the constants of both authors.

The present investigation has shown that methyl α-glucuronide was hydrolyzed at about one-half the rate of methyl α-glucoside. This, of course, varies somewhat due to the difference in activation energy. Nakano and Rånby also found that methyl α-glucuronide was hydrolyzed at about one-half the rate of methyl α-glucoside. Therefore, this apparent agreement shows that, even though Nakano and Rånby and this author disagree on actual values of rate constants, the data of each experimenter are internally consistent.

Figure 13 shows a comparison of the rate constants for methyl α-glucoside hydrolysis determined by Nakano and Rånby and by this author with those obtained by several independent investigators. This comparison utilizes the relationship between the rate constant and the Hammett acidity function suggested for methyl α-glucoside hydrolysis by Bunton, et al. (4). Hammett acidity functions used are from the tables of Paul and Long (7). It is evident from Fig. 13 that the rate constant for methyl α-glucoside found in this investigation is in good agreement with published results. This, therefore, enhances the reliability of the rate constants for methyl α-glucuronide hydrolysis found in this investigation.

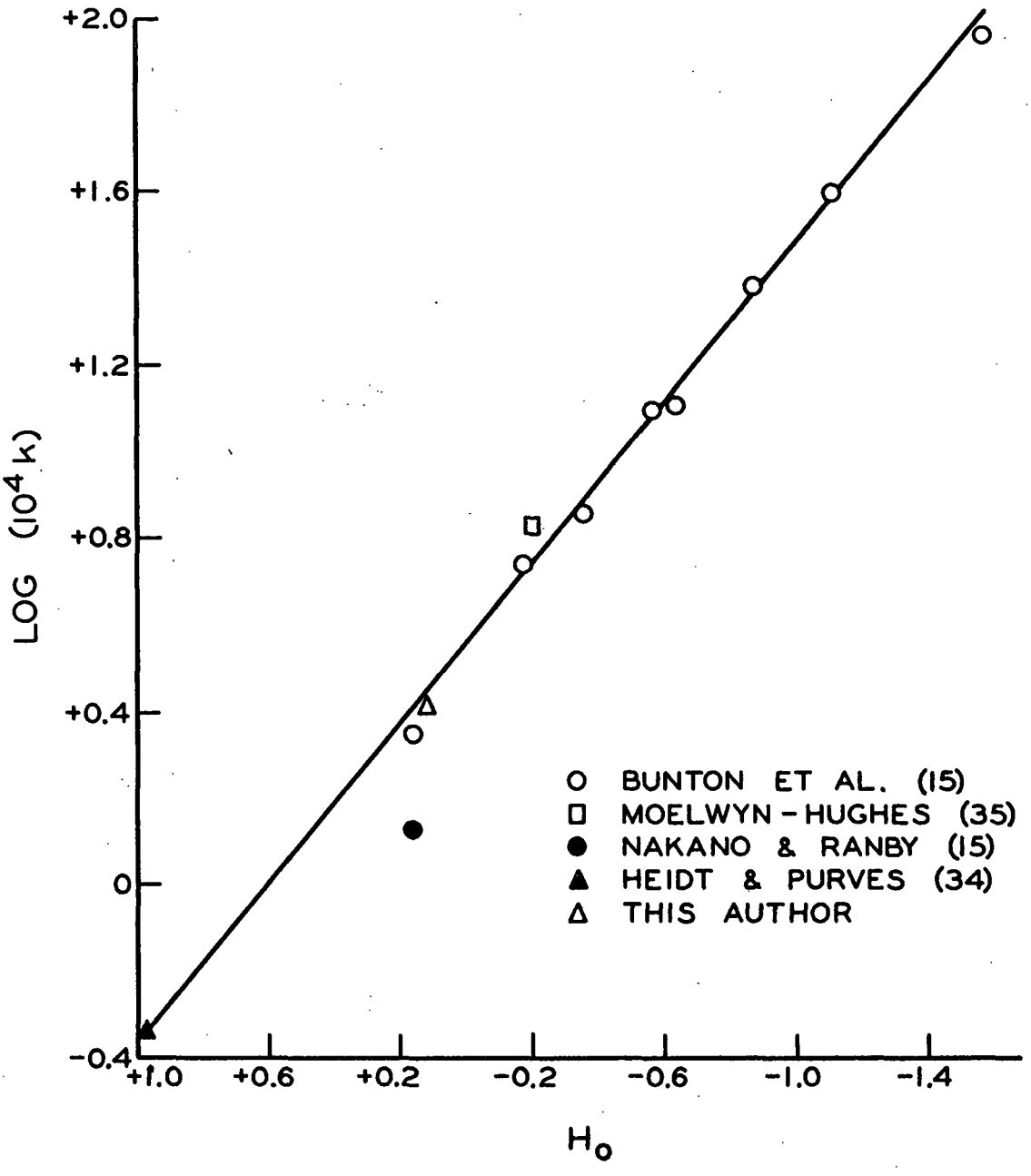

Figure 13. Comparison of Rates of Hydrolysis of Methyl α-Glucoside at 72.9°C.

ACID DEGRADATION AND THE REACTION PATHWAY

Products of the acid degradation of methyl α-glucuronide and glucuronic acid in normal sulfuric acid at 90 ± 0.1°C. were determined as furfural by measurement of optical density at 277 mμ. Results of these studies are plotted in Fig. 14 and 15.

Glasstone (36) has presented the following equations for the study of consecutive reactions.

$$\underline{A} \xrightarrow{\underline{k}^1} \underline{B} \xrightarrow{\underline{k}^2} \underline{C} \qquad (1)$$

$$\underline{c}_{\underline{B}} = \underline{a}\underline{k}_1/\underline{k}_2-\underline{k}_1 \left[\exp\left(-\underline{k}_1\underline{t}\right) - \exp\left(-\underline{k}_2\underline{t}\right)\right] \qquad (2)$$

$$\underline{dc}_{\underline{C}}/\underline{dt} = \underline{k}_2\ \underline{c}_{\underline{B}} \qquad (3)$$

In these equations $\underline{k}_1$ and $\underline{k}_2$ are the two rate constants, $\underline{a}$ is the concentration of reactant $\underline{A}$ at the beginning, and $\underline{c}_{\underline{A}}$, $\underline{c}_{\underline{B}}$, and $\underline{c}_{\underline{C}}$ are the values for $\underline{A}$, $\underline{B}$, and $\underline{C}$, respectively after time $\underline{t}$.

The degradation of methyl α-glucuronide in dilute acid was hypothesized to follow Equation (1), in which $\underline{A}$ is methyl α-glucuronide, $\underline{B}$ is glucuronic acid, and $\underline{C}$ represents degradation products as furfural. An indication of the conformity of the methyl α-glucuronide system to the hypothesis was obtained by the use of Glasstone's equations, though the following assumptions appeared necessary: (a) There were no significant rate-influencing interactions between methyl α-glucuronide and glucuronic acid under the conditions of this study. (b) The plot in Fig. 14 is sufficiently linear in the vicinity of 1000 seconds to be used in calculating a first-order rate constant.

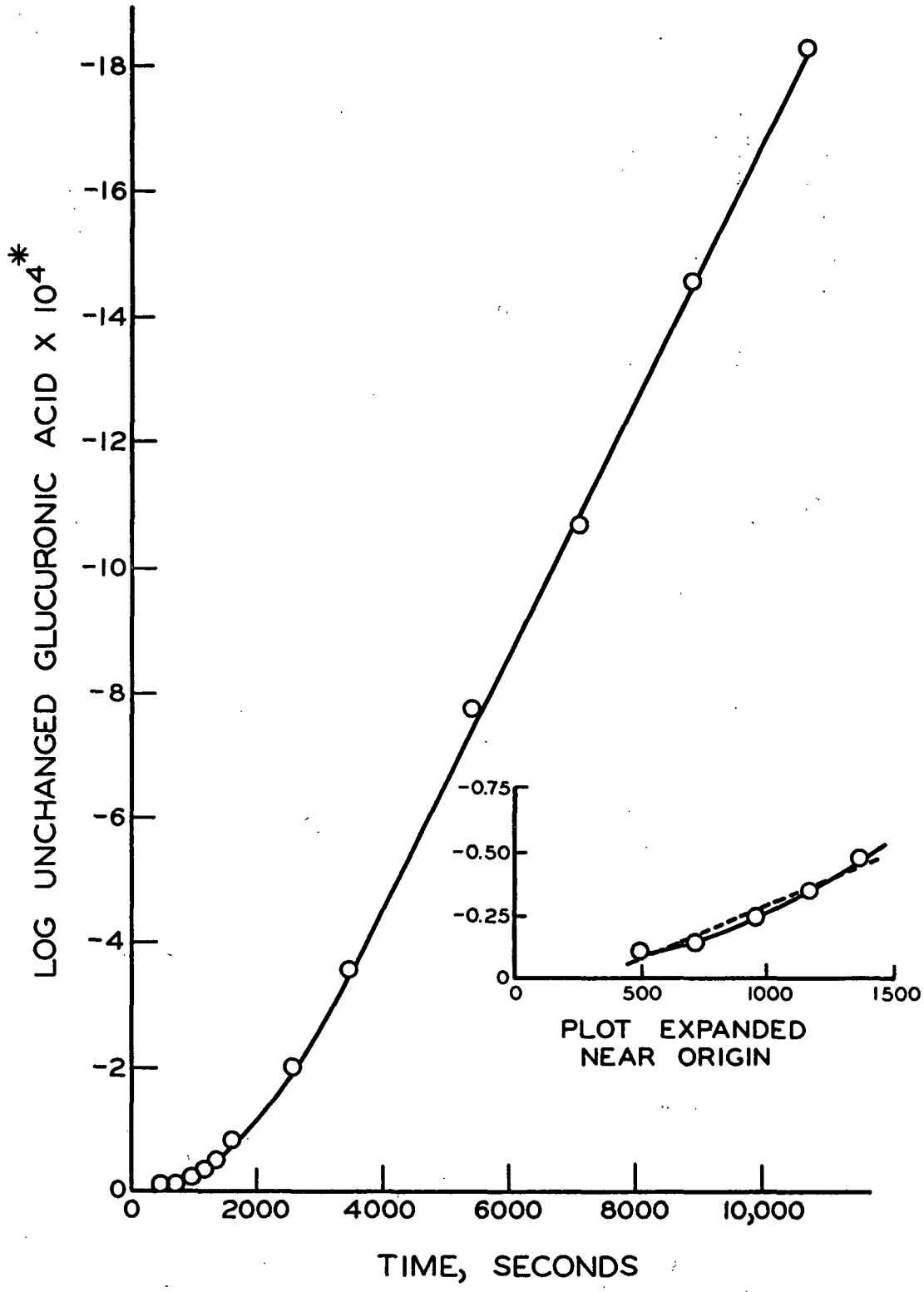

Figure 14. Acid Degradation of Glucuronic Acid

* Calculated from data from spectrophotometric furfural determinations, assuming the furfural was equivalent to reacted glucuronic acid. See Appendix II.

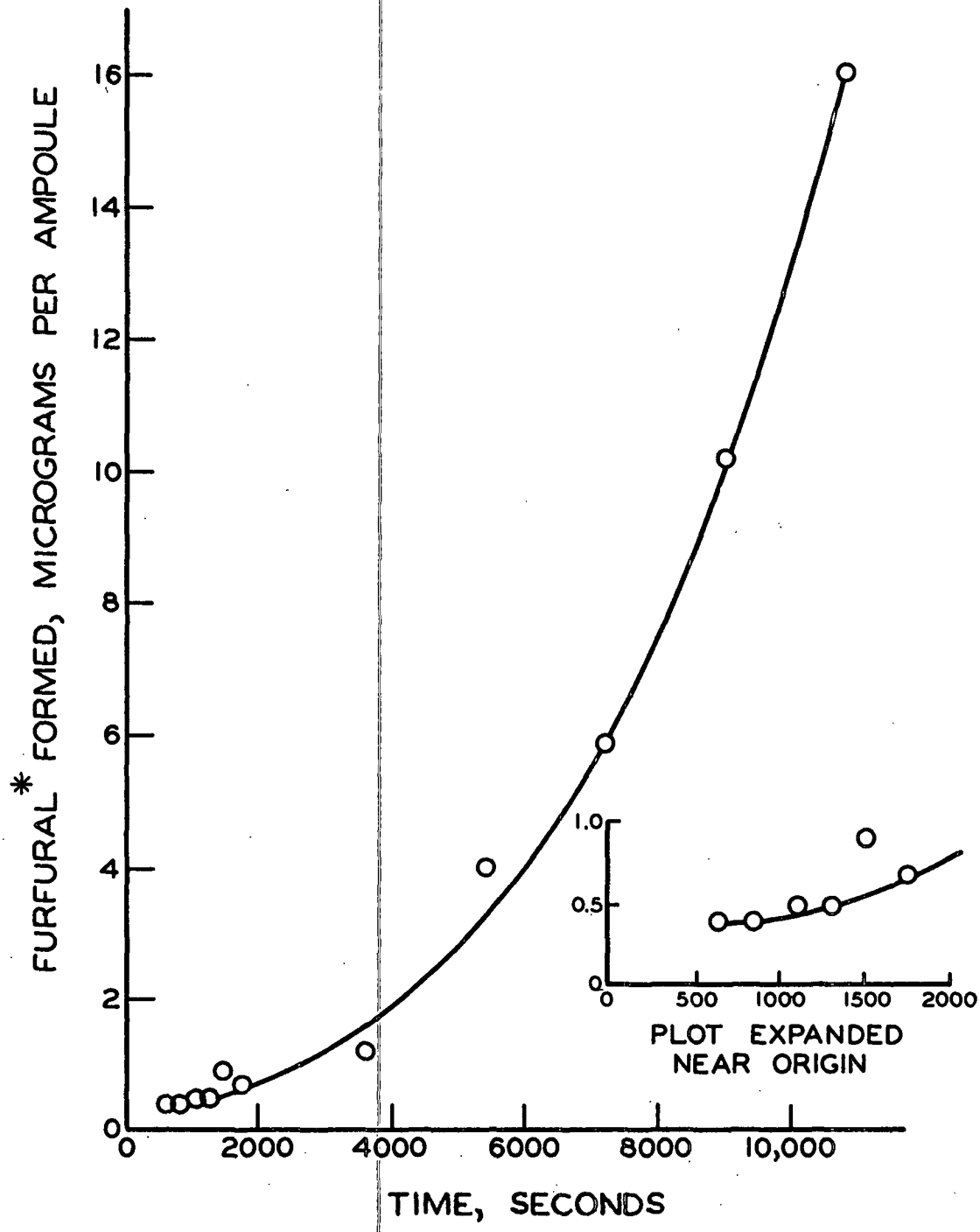

Figure 15. Acid Degradation of Methyl α-Glucuronide

*Furfural was determined spectrophotometrically at 277 mμ.

From hydrolysis studies k_1 was found to be 2.42×10^{-5} sec.$^{-1}$. From the best straight line through the initial five points of Fig. 14 k_2 was found to be 1.01×10^{-7} sec.$^{-1}$, and a was known to be 0.241 molar. Substitution in Equations (2) and (3) for the proposed two-step degradation of methyl α-glucuronide in normal sulfuric acid at 90 ± 0.1°C. yielded a calculated rate of furfural formation at 1000 seconds of 5.86×10^{-10} moles per second. From the slope at 1000 seconds in Fig. 15 an observed rate of 6.76×10^{-10} moles per second was found.

The agreement, within probable experimental error, of the calculated and observed rates indicates that the pathway proposed by Equation (1) describes the system during the portion of the reaction that was studied. This greatly reduces the possibility of significant amounts of direct degradation of methyl α-glucuronide concomitant with hydrolysis. Therefore, the predominant reaction that occurred initially when methyl α-glucuronide was treated with normal sulfuric acid at 90°C. was simple glycoside hydrolysis.

The shapes of the curves obtained in this investigation support the above indication. The linearity of Fig. 6-8 suggests simple hydrolysis, and the increasing slope of Fig. 15 would be expected for the yield of a product from the second of two consecutive reactions.

Figure 16 shows further evidence that the proposed reaction pathway describes the system studied. The figure illustrates chromatographic results of a preliminary acid hydrolysis of methyl α-glucuronide hydrazide. It may be noted that the hydrazine was rapidly split off to yield methyl

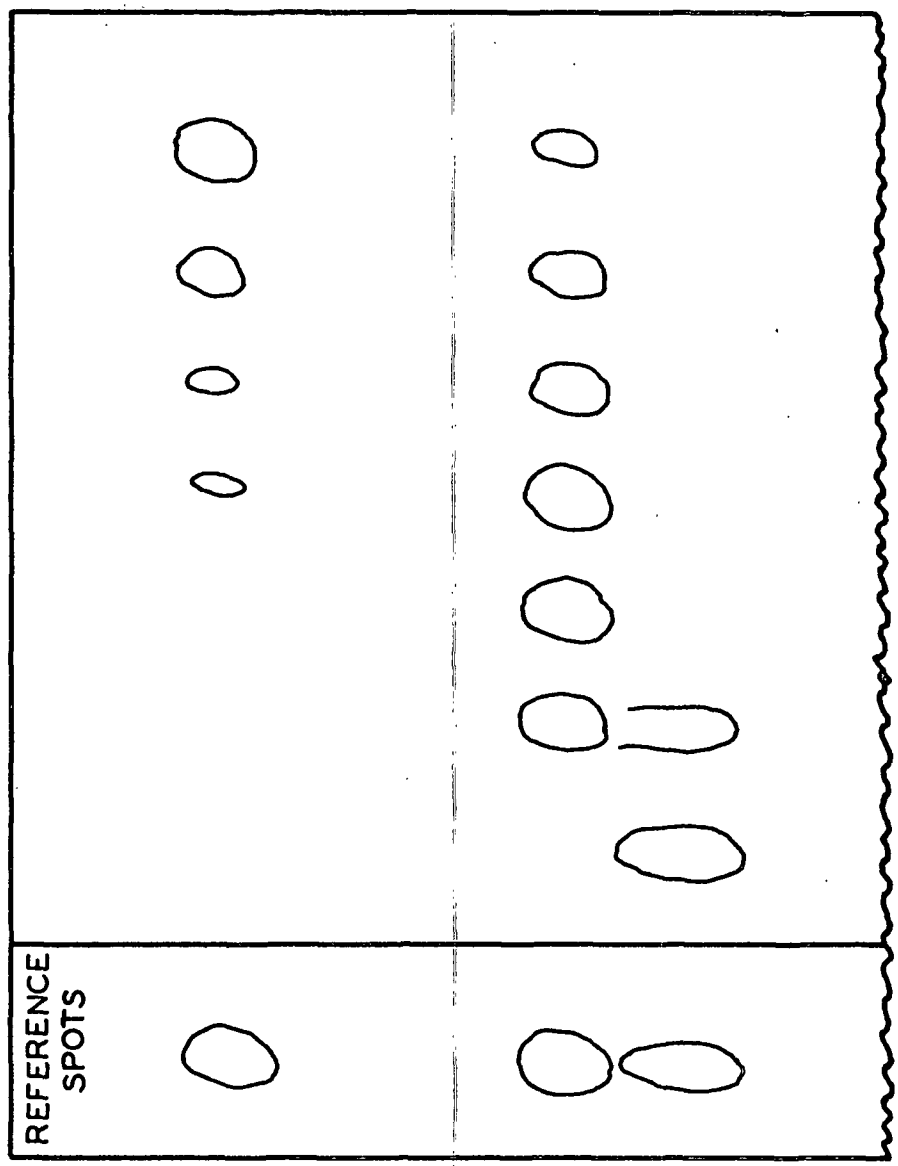

Figure 16. Chromatographic Results

α-glucuronide; the glucuronide was then hydrolyzed to glucuronic acid. That nothing else appeared on the chromatograms is evidence that no significant amounts of other carbohydrate materials were formed.

In order to visualize better the above interpretation of results, a hypothetical (and improbable) side reaction may be considered. If this side reaction produced a compound similar to methyl glucuronide or glucuronic acid faster than the compound was degraded, that compound probably would have appeared on chromatograms. However, chromatograms showed no unidentified spots. If this hypothetical side reaction product were degraded as fast as it was made, larger quantities of degradation products should have been found. But as previously noted, the experimentation yielded the expected amount of degradation product.

In the Introduction an argument against degradation of methyl α-glucuronide concomitant with hydrolysis is presented. This argument has been hereby complemented with experimental evidence.

One additional contribution of this work involves the tacit assumption made by Nakano and Rånby (15) that degradation of glucuronic acid in their system was insignificant (page 18). The investigation of degradation in the present study makes their assumption valid.

CONCLUSIONS

THE GENERAL NATURE OF HYDROLYSIS RESISTANCE OF URONOSIDES

Methyl α-glucuronide has been found not to be extremely resistant to acid hydrolysis in comparison with a similar unoxidized compound, methyl α-glucoside. This contrasts with the behavior of the aldobiouronic acid described in the Introduction. Therefore, it is evident that the resistance to hydrolysis of the aldobiouronic acids is not exhibited by all uronosides. Moreover, because both "resistant" and "nonresistant" uronosides must contain a carboxyl group, this carboxyl group cannot be the sole source of the stability of those uronosides that are resistant to hydrolysis.

Because the "resistant" and "nonresistant" uronosides differ only in the nature of their aglycon (neglecting the 4-O-methyl group on the aldobiouronic acid), it would appear logical that the resistance of the aldobiouronic acids should be due to some as yet unexplained interaction between carboxyl and aglycon.

THE DIFFERENCE IN RATES OF HYDROLYSIS OF METHYL α-GLUCURONIDE AND METHYL α-GLUCOSIDE

THE PROPOSED INDUCTIVE EFFECT

Though objections can be raised to the application of the theory of absolute reaction rates to reactions in solution, the trends found in this investigation and in the literature, which will be discussed in light of this theory, appear to be quite pronounced. This discussion involves

differences in quantities (energies of activation, entropies of activation) determined for two systems that are almost identical. Therefore, deviations from the requisites for application of the absolute theory might be quite similar for both systems described.

In this discussion of the inductive effect it is necessary to assume that methyl α-glucuronide hydrolyzes *via* the mechanism suggested for methyl glucoside hydrolysis by Bunton, *et al.* (4) (page 6). This is necessary because the original proposals of a significant inductive effect in uronoside hydrolysis were tacitly based on this assumption. Novikova and Konkin's (10) finding that the Bunton theory applies to other glycosides adds credibility to the assumption.

Relationships between the electronic theories of organic reactivity, the influence of substituents on reactants, and activation energies were investigated by Hinshelwood, Laidler, and Timm (37). They concluded that the changes in reactivity which result from electronic displacements caused by substituents are due primarily to changes in the activation energy. They pointed out that an inductive effect will influence the repulsive energies and bond strengths of the groups involved in a reaction and, thereby, influence the activation energy.

Marchessault and Rånby (12) and Nakano and Rånby (15) described uronoside hydrolysis in terms of an electronic theory and concluded that an inductive effect of the carboxyl group strengthens the glycosidic bond. Hamilton and Thompson (13) described the proposed inductive effect as acting in a manner that would increase repulsion between the uronoside and

an attacking hydronium ion. It is therefore quite evident that the inductive effects proposed for uronoside hydrolysis have been considered to act in a manner that, according to the arguments of Hinshelwood, Laidler, and Timm, would influence the energy of activation.

In a case where the inductive effect has been supposed to retard reaction, as in uronoside hydrolysis, the activation energy should be larger than for reaction of a compound that does not have a substituent capable of producing a stabilizing inductive effect. Nakano and Rånby attributed the slightly slower hydrolysis of methyl α-glucuronide than methyl α-glucoside to a stabilizing inductive effect caused by the uronic carboxyl group. However, the present investigation has shown that the activation energy for methyl α-glucuronide hydrolysis was actually less than that for hydrolysis of methyl α-glucoside. By the arguments presented above, this result indicates that there was not a significant stabilizing inductive effect acting in the hydrolysis of methyl α-glucuronide.

The observations of this investigation quite possibly could represent a definite characteristic of methyl uronoside behavior, because Morell and Link (14) have found a similar effect, i.e., a lower energy of activation for the hydrolysis of methyl α-galacturonide than for methyl α-galactoside.

A consideration of the structure of methyl α-glucuronide can lead to further reasons why a stabilizing inductive effect should not be influential in hydrolysis. The proponents of the inductive effect have assumed that hydrolysis proceeds through the cyclic intermediate in the Bunton theory. According to this mechanism the point influenced by the inductive effect,

the oxygen of the glycosidic bond, lies three atoms away from the carboxyl group. Alexander (38) has noted that the inductive effect in aliphatic systems becomes almost negligible at a distance of three atoms away from the primary electron attractor. Therefore, an inductive effect as proposed appears somewhat improbable from the standpoint of distance alone.

Alexander (38) also pointed out that a carbon-oxygen (single) bond has a low polarizability. In a discussion of inductive effects Smith and Eyring (39) described polarizability as a ". . . measure of the ease of transferring electronic charge from one atom to an adjacent one." Therefore, in addition to distance, the inductive effect might be diminished by the presence of the ring oxygen between the carboxyl and the glycosidic bond.

THE ENTROPY OF ACTIVATION

Because this investigation was not designed to determine mechanisms, possible reasons given here for the differences in hydrolysis behavior of methyl α-glucuronide and methyl α-glucoside must be purely speculative in nature. Calculated entropies of activation in Table V are an aid in interpretation of these differences. Use of the theory of absolute reaction rates in calculation of these values is outlined in Appendix IV.

The entropies of activation provide evidence against one possible theory of the behavior of methyl α-glucuronide. In this theory the acyclic pathway of Bunton's mechanism is assumed. Protonation of the ring oxygen would occur, followed by a shift of electrons promoted by a possible

activating inductive effect. By the arguments of Hinshelwood, Laidler, and Timm this mechanism will account for the lower activation energy of methyl α-glucuronide. However, the mechanism would not be compatible with the entropies of activation shown on Table V. Because of the proposed ring opening in this mechanism, methyl α-glucuronide would be expected to have an entropy of activation similar to that of methyl α-glucoside.

TABLE V

CALCULATED THERMODYNAMIC CONSTANTS OF ACTIVATION FOR HYDROLYSIS AT 80 ± 0.1°C.

	Methyl α-Glucuronide	Methyl α-Glucoside
$\Delta H^{\ddagger}$	30,900 cal./mole	34,900 cal./mole
$\Delta S^{\ddagger}$	6.42 e.u.	18.9 e.u.
$\Delta F^{\ddagger}$	28,600 cal./mole	28,200 cal./mole

From a comparison of rate constants, activation energies, and entropies of activation it is evident that the lower rate constant for methyl α-glucuronide hydrolysis resulted from the lower entropy of activation. In view of this apparent importance of the entropy of activation, the entropy differences in Table V provide the logical basis for further consideration of the differences in the hydrolysis of methyl α-glucoside and methyl α-glucuronide.

Shafizadeh (3) has presented strong arguments in favor of the acyclic intermediate in the Bunton mechanism (page 6) occurring in glycoside hydrolysis, and Richards (40) has done likewise in favor of the cyclic intermediate. Thus, it would appear that different glycosides may hydrolyze by different intermediates. In the formation of the activated state,

methyl α-glucoside underwent a rather substantial positive entropy of activation relative to that of methyl α-glucuronide, which implies a greater increase in freedom. The difference in the entropies of activation of the two compounds suggests that the mechanisms of their hydrolysis differ in some manner. However, because a substantial increase in freedom can be accounted for by either pathway of the Bunton theory, specific mechanisms or intermediates of that theory cannot be assigned to the hydrolysis of methyl α-glucoside or methyl α-glucuronide.

Of no small influence on this discussion are results calculated from the data of Morell and Link (14). These results, which are very similar to those found in the present investigation, show the entropy of activation for methyl α-galacturonide hydrolysis to be 2.78 e.u. and for methyl α-galactoside, 20.7 e.u.

SUMMARY

In order to gain a better understanding of the resistance of uronosides to hydrolysis, the study of hydrolysis of methyl α-D-glucopyranosiduronic acid (methyl α-glucuronide) has been undertaken. Because acid degradation has been found to occur in uronoside systems undergoing hydrolysis, the significance and point of origin of this degradation was determined.

The first objective of this investigation was the synthesis of an amount of pure methyl α-glucuronide sufficient for completion of the hydrolysis studies planned. Catalytic air oxidation of methyl α-D-glucopyranoside (methyl α-glucoside) yielded a crude product which was purified by conversion into crystalline methyl α-glucuronide hydrazide. This hydrazide provided the first proof by means of a crystalline derivative that the catalytic air oxidation of methyl α-glucoside produced methyl α-glucuronide. Barium hydroxide was used to hydrolyze the hydrazine off the methyl α-glucuronide hydrazide and form the pure barium salt of methyl α-glucuronide. Prior to hydrolysis studies, the barium was removed from the salt on an ion-exchange resin.

These preparations mark the first synthesis of the pure barium salt of methyl α-glucuronide and the first synthesis of pure methyl α-glucuronide without chromatography. This also is the first demonstration of the lability to basic hydrolysis of the hydrazine portion of methyl α-glucuronide hydrazide.

The initial portions of the acid hydrolysis of methyl α-glucuronide and methyl α-glucoside were studied by measurement of the methanol evolved. Hydrolyses were run in normal sulfuric acid at 70, 80, and 90 ± 0.1°C. Rate constants and energies of activation were determined. The activation energy for methyl α-glucuronide hydrolysis is in agreement with one determined from the recent optical rotation observations of Nakano and Rånby. The energy of activation for methyl α-glucoside hydrolysis agrees with the most reliable published value. Though this investigation and the work of Nakano and Rånby have shown a similar ratio between the rate constants for hydrolysis of methyl α-glucuronide and methyl α-glucoside, the actual values of the rate constants differed by factors of from two to three.

In order to compare these rate constants with values in the literature, a plot of log k vs. Hammett acidity function was used. All available rate constants for methyl α-glucoside hydrolysis at 72.9°C., interpolated in many cases, were plotted. The data, except that of Nakano and Rånby, form the straight line with slope near unity that would be expected from Bunton's theory of methyl glucoside hydrolysis. It may therefore be concluded that this investigation has produced reliable data, and these data indicate that methyl α-glucuronide does not possess extraordinary resistance to acid hydrolysis.

Because it has become evident that there are both "resistant" and "nonresistant" uronosides, the uronic carboxyl group cannot be the sole source of the stability of those uronosides that are resistant to hydrolysis.

Degradation concomitant with uronoside hydrolysis was studied by determination of furfural. The rate of formation of furfural from glucuronic acid was found to be about 240 times slower than the rate of hydrolysis of methyl α-glucuronide. Calculations based on rates of formation of furfural from glucuronic acid and from methyl α-glucuronide support the reaction sequence: hydrolysis of methyl α-glucuronide followed by degradation of glucuronic acid.

Nakano and Rånby attributed the slightly slower hydrolysis of methyl α-glucuronide than methyl α-glucoside to a stabilizing inductive effect. From use of the theory of absolute reaction rates in a study of inductive effects, Hinshelwood, Laidler, and Timm concluded that the changes in reactivity which result from electronic displacements caused by substituents are due primarily to changes in the activation energy. Recognition of the parallelism between their conclusion and the mechanism by which the carboxyl group has been proposed to stabilize uronosides has led to an expectation of a higher energy of activation for methyl α-glucuronide hydrolysis than for methyl α-glucoside. Because the reverse of this expectation was found for methyl α-glucuronide vs. glucoside in this investigation and methyl α-galacturonide vs. galactoside in the literature, it is concluded that methyl α-glucuronide is probably not stabilized by an inductive effect.

Because this investigation was not designed as a mechanism study, mechanistic conclusions must be speculative. It has been observed that the lower rate constant for methyl α-glucuronide hydrolysis resulted from the lower entropy of activation of methyl α-glucuronide. The difference

in the entropies of activation of methyl α-glucoside and methyl α-glucuronide suggests that the mechanisms of their hydrolysis differ in some manner, but knowledge of the systems studied is insufficient to provide a basis for description of the hydrolysis of the two compounds in terms of specific mechanisms or intermediates.

ACKNOWLEDGMENT

The author wishes to acknowledge gratefully the assistance and suggestions of the thesis advisory committee: E. E. Dickey, J. W. Green, and D. G. Williams. The author also wishes to express appreciation to B. G. Rånby for a preprint of his paper and samples of the potassium salts of methyl α- and β-glucuronide for chromatography.

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APPENDIX I

EXPERIMENTAL PROCEDURES

SYNTHESIS OF METHYL α-GLUCURONIDE

CATALYTIC AIR OXIDATION

Fifty-eight grams of methyl α-glucoside were treated by the catalytic air oxidation procedure of Mehltretter, et al. (26). The methyl α-glucoside was put in a 3-liter Morton (creased) flask with 7.6 g. of platinum-Darco G-60 catalyst and 6.3 g. of sodium bicarbonate. The reaction mixture, stirred at about 240 r.p.m., was maintained at 50°C. by a water bath as air was bubbled into the mixture at about 290 liters per hour. Additional sodium bicarbonate was added as needed to control pH between 7.5 and 8.5. When the reaction reached 80% completion, as determined by the amount of bicarbonate added, it was stopped, and the reaction mixture was filtered. Sodium ions were removed on a column of Amberlite IR-120(H) resin; then the solution was neutralized with barium hydroxide. The solution was concentrated to about 250 ml. and placed in the refrigerator for several days during which time insoluble barium salts of impurities precipitated.

Barium ions were removed from small amounts of the above material on IR-120(H) resin, and samples of the solution and the precipitate were examined by paper chromatography. The developer used was ethyl acetate-pyridine-water-acetic acid (5:5:3:1). Spots were detected by dipping the sheet in 3% silver nitrate in water-acetone (1:19), spraying with 0.5N ethanolic sodium hydroxide, and finally, after 10 minutes, dipping in 10% aqueous sodium thiosulfate. Another procedure for detection of certain

impurity spots required a spray of 0.4% 2,4-dinitrophenylhydrazine in 2N hydrochloric acid followed by a spray of 0.5N ethanolic sodium hydroxide.

After allowing precipitation of impurities to occur for several days (and filtering off the precipitate each day), the oxidation product as barium salt was concentrated to about 100 ml. and run in a fine stream from a pipet into about 500 ml. of rapidly stirred absolute ethanol. This precipitated the crude barium salt of methyl α-glucuronide and left the unreacted methyl α-glucoside in solution. The resulting white floc, after drying in a vacuum oven at 50°C., weighed 73 grams. Yield was 85.7%. Analysis of the product showed: methoxyl, 10.8%; barium, 26.5%. Methoxyl content was determined by Institute Method 18. The barium determination was described by Smith and Shriner (42).

HYDRAZIDE PREPARATION

The crude product as barium salt was dissolved in about 500 ml. water. Normal sulfuric acid was added to precipitate the barium as barium sulfate and lower the pH to 2-2.5. After filtration, the solution was stirred with IR-120(H) resin to remove the remaining barium. The solution was then concentrated to about 60 ml. In order to remove water, the sirup was concentrated from about 400 ml. absolute ethanol seven times to yield a dry sirup of crude methyl α-glucuronide weighing 54.6 g. The yield over this step was 102.6%, indicating that some alcohol remained; accumulative yield, 87.8%.

The sirup was dissolved in 910 ml. of methanol in preparation for diazomethane esterification by an adaptation of the method of Hardegger and Spitz (28). Ethereal diazomethane was prepared by the method of

DeBoer (43). The methanolic solution to be esterified was cooled to 0°C. in a dry ice-acetone bath. Ethereal diazomethane was added until the solution became yellow, indicative of excess diazomethane. The temperature was controlled between -1 and +1°C. during this exothermic reaction by adding dry ice to the acetone bath between the incremental additions of diazomethane. The diazomethane added amounted to 170% of the theoretical requirement. Stirring of the solution was continued for two hours. The solution was then concentrated to a thick sirup to remove ether and excess diazomethane; next the sirup was diluted to 140 ml. of methanolic solution.

The method of Wolfrom, Kowkabany, and Binkley (27) was used for conversion of the ester into the hydrazide. The methanolic solution of the ester was added slowly with stirring to 37.2 ml. of 95+% hydrazine in 118 ml. methanol at room temperature. Within 15 minutes the solution became a mass of crystals. The reaction mixture was allowed to stand for 30 minutes before the crystals were filtered off and dried in a vacuum oven at 50°C. The yield of this hydrazide was 27.5 g. or 47.2%. Accumulative yield was 41.4%. No appreciable amounts of product crystallized from the mother liquor of the reaction after it stood for several days.

After several recrystallizations from water-ethanol, the yield of methyl α-glucuronide hydrazide had decreased to 15.5 g. or 56.3%. Accumulative yield had been reduced to 23.4%. The product melted at 231.5-232.5°C. with a specific optical rotation of +150° (c, 0.623 in water).

HYDROLYSIS OF THE HYDRAZIDE WITH BARIUM HYDROXIDE

Utilized here was an adaptation of the Fischer and Passmore procedure (32). The 15.5 g. of hydrazide were added to a boiling solution of 50 g. crystalline barium hydroxide in 1000 ml. water. After allowing this to reflux for 10 minutes, the heat was removed and normal sulfuric acid was used to lower the pH to 7.5. The solution was filtered, allowed to cool, and then shaken for two half-hour periods with IR-120(H) resin to remove liberated hydrazine.

Barium hydroxide solution was used to raise the pH of the solution to 7.6. Then the solution was concentrated to 30 ml. of clear, colorless sirup. This was run from a pipet into about 200 ml. of stirred absolute ethanol. The resulting white floc was filtered and dried. The product, the barium salt of methyl α-glucuronide, weighed 18.3 g.; yield, 92%. Over-all yield, based on the original methyl α-glucoside, was 21.5%.

Any occluded alcohol was removed by drying a small sample of an aqueous sirup of the purified product in an Abderhalden drier over boiling acetone. Analysis of the purified product showed: methoxyl, 11.00%; barium, 23.96%.

ACID HYDROLYSIS AND DEGRADATION OF METHYL α-GLUCURONIDE

HYDROLYSIS OF METHYL α-GLUCURONIDE

Sample Preparation

A portion of the barium salt of methyl α-glucuronide, 8.3626 g., was dissolved in 100 ml. of water and put through a column of IR-120(H) to

remove the barium. After concentration of the free acid solution, a weighed portion was potentiometrically titrated. The result of the titration showed that this master solution had a concentration of 0.07572 g. methyl glucuronide per milliliter or per 1.0217 g. solution. From this master solution, 0.241 molar solutions of methyl α-glucuronide in normal sulfuric acid were prepared for each hydrolysis run.

A modified hypodermic syringe was used to load the solution for hydrolysis into two-milliliter glass ampoules. The modification involved a stop on the syringe plunger that enabled the syringe to deliver reproducible amounts. Eight or nine ampoules were loaded for each hydrolysis run. The first and last ampoules and one in the middle of the series were tared before loading. Ampoule loads of two grams were found to vary by only a few tenths of a milligram. After loading, the ampoules were sealed in a gas-oxygen flame. The flame was small enough not to heat the ampoule contents.

Hydrolysis Procedure

Hydrolyses were conducted in an ethylene glycol bath maintained at 70, 80, or 90 ± 0.1°C. Bath temperatures were measured by a calibrated thermometer. The ampoules, weighted with lead collars, were lowered in a basket into the bath. At various times individual ampoules were removed from the bath and quenched by immersion in an ice-water mixture.

Preparation of Samples for Analysis

A 10-ml. distilling flask was clamped in an upright position, and 1.5 ml. water were put in a small beaker nearby. An ampoule was opened

and its contents transferred to the distilling flask by means of a capillary pipet. To make the transfer quantitative, the 1.5 ml. water was pipetted in increments into the ampoule and from there into the distilling flask. The acid in the distilling flask was then neutralized by addition of the calculated amount of sodium hydroxide solution.

The arrangement for distillation of the methanol liberated on hydrolysis is shown in Fig. 17. A multiple hot plate arrangement was used so that up to six distillations could be run simultaneously.

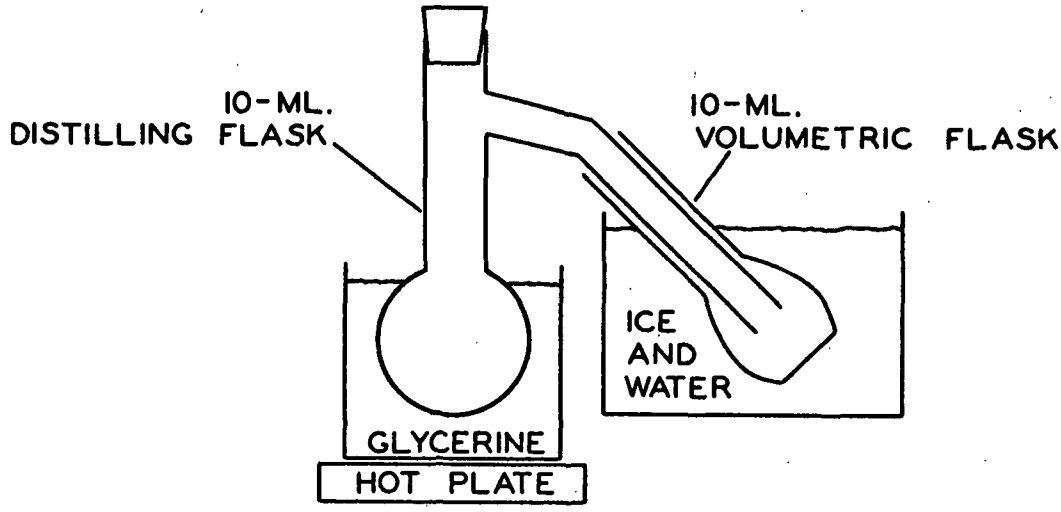

Figure 17. Semimicro Distillation Apparatus

The contents of the distilling flask were distilled almost to dryness. This required two to three hours. After distillation, water was used to wash down the side arm of the distilling flask so that the washings ran into the volumetric flask; then the volumetric flask was filled to the mark.

Methanol Determination

The procedure of Boos (33) was used without modification. In this method potassium permanganate and phosphoric acid oxidized methanol to formaldehyde. The formaldehyde reacted with 4,5-dihydroxy-2,7-naphthalene-disulfonic acid (chromotropic acid) to give color measured at 580 mμ on the Beckman DU spectrophotometer.

HYDROLYSIS OF METHYL α-GLUCOSIDE

The methyl α-glucoside used in this study was a commercial preparation which had been recrystallized once from methanol and once from ethanol. It melted at 166°C. and had a specific optical rotation of +157.5° (c, 1.14 in water). For methyl α-glucoside, Bollenback (44) reported m. 167°C. and $[\alpha]_D$ +158.2° (c, 1.0 in water). The recrystallization from ethanol was necessary to avoid a large "blank" methanol determination from unhydrolyzed methyl α-glucoside.

Methyl α-glucoside hydrolysis studies were conducted in the same manner as were the studies of methyl α-glucuronide hydrolysis. The concentration of methyl α-glucoside in the solutions for hydrolysis was 0.257 molar.

ACID DEGRADATION OF METHYL α-GLUCURONIDE

Samples were prepared and reacted as has been described for methyl α-glucuronide hydrolysis. After reaction, the ampoules were opened and their contents transferred to 10-ml. volumetric flasks. The flasks were filled to the mark; the contents were not neutralized. Furfural in these

solutions was then determined by measurement of the optical density at 277 mμ on the Beckman DU spectrophotometer. This determination was described by Wolfrom, Schuetz, and Cavalieri (18).

ACID DEGRADATION OF GLUCURONIC ACID

A commercial sample of glucuronic acid was used. It melted at 160°C. and had a specific optical rotation of +35.7° (c, 5.06 in water) after mutarotation. For glucuronic acid, Artz and Osman (45) reported m. 165°C. and $[\alpha]_D$ +36°. The solution for the degradation study was prepared with a glucuronic acid concentration of 0.234 molar. The procedures used in this investigation were identical with those used in the study of methyl α-glucuronide degradation.

APPENDIX II

EXPERIMENTAL RESULTS

HYDROLYSIS OF METHYL α-GLUCURONIDE

Temperature: 70 ± 0.1°C.

Time, sec.	mg. Methanol per Ampoule	Equivalent mg. Methyl Glucuronide Hydrolyzed, x	$\log[(a-x)/a]$
7,978	0.246	1.60	-0.0070
10,608	0.320	2.08	-0.0092
13,754	0.385	2.50	-0.0110
16,352	0.475	3.09	-0.0137
18,940	0.529	3.44	-0.0152
22,069	0.633	4.12	-0.0183

a = 99.7 mg. Methyl glucuronide per ampoule at start of hydrolysis.

The amounts of methyl glucuronide are equivalent to the amounts of methanol in the second column. The fraction hydrolyzed is the mg. methyl glucuronide divided by a. This fraction subtracted from 1 gives $(a-x)/a$, the unreacted fraction.

Temperature: 80 ± 0.1°C.

Time, sec.	mg. Methanol per Ampoule	Equivalent mg. Methyl Glucuronide Hydrolyzed, x	$\log[(a-x)/a]$
1776	0.180	1.17	-0.0052
2763	0.300	1.95	-0.0086
3547	0.362	2.35	-0.0104
4434	0.462	3.00	-0.0133
5325	0.555	3.61	-0.0160
6250	0.645	4.19	-0.0187

a = 99.5 mg. Methyl glucuronide per ampoule at start of hydrolysis.

Temperature: 90 ± 0.1°C.

Time, sec.	mg. Methanol per Ampoule	Equivalent mg. Methyl Glucuronide Hydrolyzed, x	log[(a-x)/a]
632	0.186	1.21	-0.0054
839	0.273	1.78	-0.0078
1084	0.325	2.11	-0.0093
1293	0.425	2.76	-0.0122
1492	0.520	3.38	-0.0150
1743	0.582	3.78	-0.0168

a = 99.7 mg. Methyl glucuronide per ampoule at start of hydrolysis.

HYDROLYSIS OF METHYL α-GLUCOSIDE

Temperature: 70 ± 0.1°C.

Time, sec.	mg. Methanol per Ampoule	Equivalent mg. Methyl Glucoside Hydrolyzed, x	log[(a-x)/a]
3,636	0.179	1.09	-0.0048
5,390	0.255	1.55	-0.0068
7,162	0.352	2.13	-0.0095
9,311	0.454	2.75	-0.0122
11,050	0.522	3.16	-0.0141
12,792	0.606	3.67	-0.0164
14,884	0.708	4.29	-0.0193

a = 99.0 mg. Methyl glucoside per ampoule at start of hydrolysis.

Temperature: 80 ± 0.1°C.

Time, sec.	mg. Methanol per Ampoule	Equivalent mg. Methyl Glucoside Hydrolyzed, x	log[(a-x)/a]
1256	0.252	1.53	-0.0067
1620	0.320	1.94	-0.0086
1906	0.380	2.30	-0.0102
2212	0.420	2.55	-0.0113
2564	0.520	3.15	-0.0140

a = 99.0 mg. Methyl glucoside per ampoule at start of hydrolysis.

Temperature: 90 ± 0.1°C.

Time, sec.	mg. Methanol per Ampoule	Equivalent mg. Methyl Glucoside Hydrolyzed, x	log[(a-x)/a]
238	0.155	0.94	-0.0042
318	0.225	1.36	-0.0060
416	0.306	1.86	-0.0082
496	0.395	2.39	-0.0106
568	0.426	2.58	-0.0115
659	0.490	2.97	-0.0133
828	0.657	3.98	-0.0179

a = 98.9 mg. Methyl glucoside per ampoule at start of hydrolysis.

DEGRADATION OF GLUCURONIC ACID

Temperature: 90 ± 0.1°C.

Time, sec.	γ Furfural* per Ampoule	Equivalent γ Glucuronic Acid Degraded, x	log[(a-x)/a]
490	1.2	2.4	-0.000012
699	1.8	3.6	-0.000016
948	2.9	5.9	-0.000024
1155	3.9	7.9	-0.000036
1358	5.3	10.7	-0.000050
1612	8.4	17.0	-0.000085
2575	20.8	42.0	-0.000200
3467	36.9	74.6	-0.000358
5377	80.4	162.5	-0.000775
7069	110.4	223.1	-0.001070
8866	150.4	303.9	-0.001460
10662	189.4	382.7	-0.001834

a = 90.8 mg. Glucuronic acid per ampoule at start of reaction.

*Calculated from data from spectrophotometric furfural determinations, assuming the furfural found was equivalent to reacted glucuronic acid.

DEGRADATION OF METHYL α-GLUCURONIDE

Temperature: 90 ± 0.1°C.

Time, sec.	γ Furfural* per Ampoule
632	0.4
840	0.4
1084	0.5
1292	0.5
1497	0.9
1744	0.7
3607	1.2
5421	4.0
7208	5.9
9000	10.2
10811	16.1

*Furfural was determined spectrophotometrically at 277 mμ.

APPENDIX III

ACID HYDROLYSIS OF METHYL α-GLUCURONIDE HYDRAZIDE

This hydrolysis study was conducted in a manner almost identical with the other hydrolyses. One necessary modification in procedure was the distillation of an extra 2 ml. of water in order to carry all methanol over into the distillate.

Because of the varying nature of the system, results from this study must be considered to be only approximations. As hydrazine was split off the methyl α-glucuronide hydrazide, hydrazine sulfate was formed. Because some of the hydrazine sulfate precipitated, a variable concentration of sulfuric acid resulted. This concentration decreased from 1.41 normal at the start of the reaction to a probable 1.12 normal after all the hydrazine had been split off the uronoside.

The results of this study are shown in Fig. 18. The first-order rate constant at 80 ± 0.1°C. determined from this plot is 0.401×10^{-3} min.$^{-1}$. This is quite similar to that obtained for methyl α-glucuronide hydrolysis at 80 ± 0.1°C., 0.413×10^{-3} min.$^{-1}$. One interpretation of this similarity is that the hydrazine portion of the molecule was split so rapidly that its presence did not significantly influence the rate of hydrolysis of the glycosidic bond. Such a conclusion would appear compatible with the chromatographic results shown in Fig. 16.

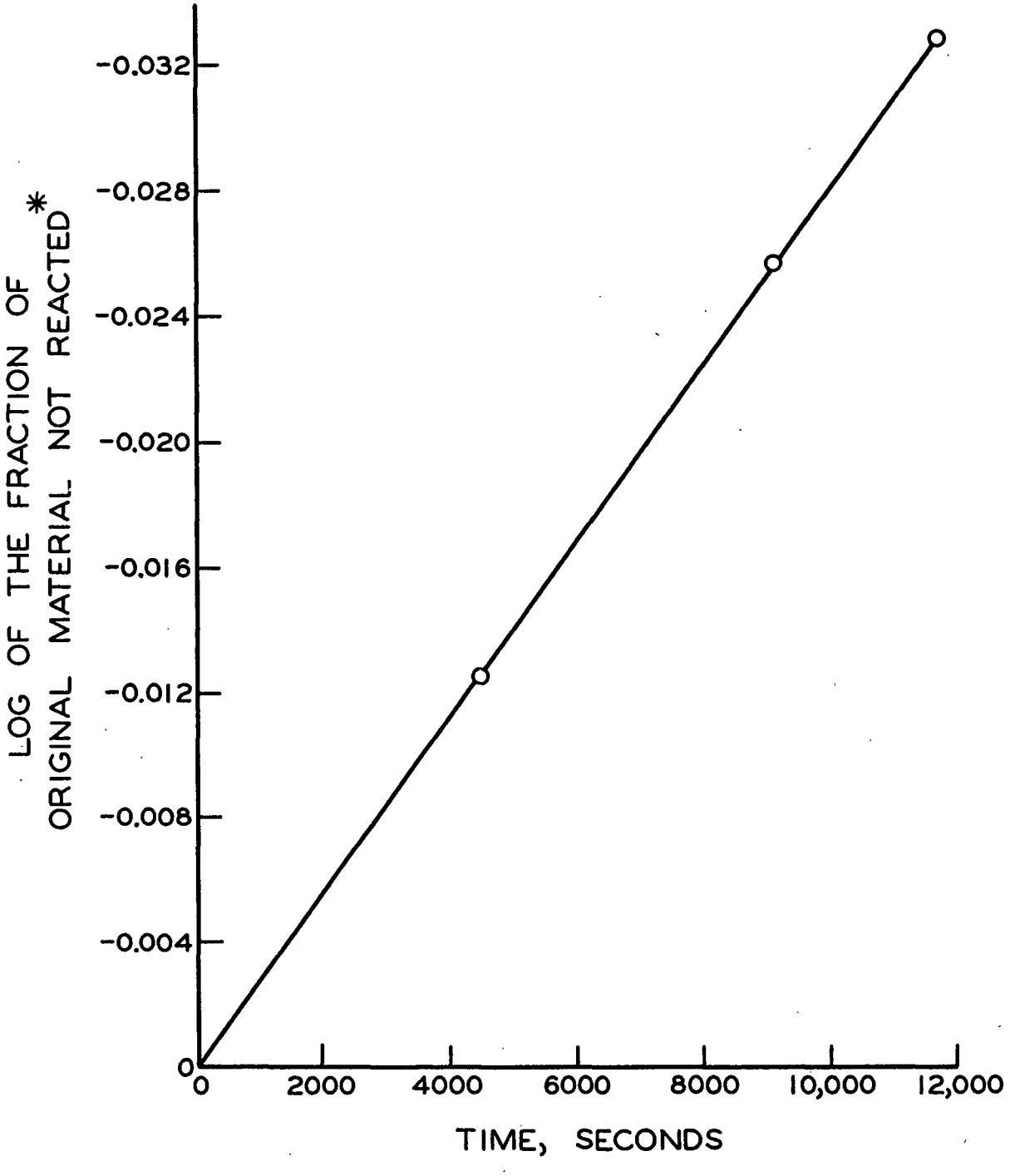

Figure 18. Hydrolysis of Methyl α-Glucuronide Hydrazide at 80 ± 0.1°C.

* Calculated from methanol evolution data as described in Appendix II. The methanol found is considered to be equivalent to the amount of methyl α-glucuronide hydrolyzed.

APPENDIX IV

CALCULATION OF THERMODYNAMIC CONSTANTS FOR HYDROLYSES

Experimental activation energies, E_{exp}, were calculated from the Arrhenius equation. In using this equation, the appropriate slope in Fig. 12, determined by the method of least squares, is $-E_{exp}/2.303\ R$.

Eyring (41) has shown that the heat of activation, $\Delta H^{\ddagger}$, may be calculated by

$$E_{exp} = RT + \Delta H^{\ddagger} - p\Delta v^{\ddagger} \qquad (1)$$

where R = the gas constant,

T = the absolute temperature,

p = pressure, and

$\Delta v^{\ddagger}$ = change in volume during reaction.

For reactions in solution $\Delta v^{\ddagger}$ is very small, so that $p\Delta v^{\ddagger}$ is approximately equal to zero.

Further use of the theory of absolute reaction rates requires consideration of the nature of the reaction studied. The hydrolysis of glycosides and uronosides may be represented as

$$G + H^{+} \longrightarrow B^{\ddagger} \longrightarrow \text{products} \qquad (2)$$

where G is the glycoside or uronoside,

H^{+} is the proton, and

$B^{\ddagger}$ is the activated complex.

According to the law of mass action,

$$\text{rate} = k_s[G][H^+] \qquad (3)$$

where k_s is the rate constant.

However, the hydrolyses of interest in the present investigation were conducted in an excess of sulfuric acid solution, so that $[H^+]$ does not change appreciably during the reaction. Therefore, Equation (3) becomes

$$\text{rate} = k_{obs.}[G] \qquad (4)$$

where $k_{obs.}$ is the pseudo first-order rate constant and is equal to $k_s[H^+]$. From Eyring's theory of absolute reaction rates (41), for reactions in the gas phase

$$\text{rate} = (kT/h)c_{\pm} \qquad (5)$$

where k = Boltzman's constant,

h = Planck's constant, and

$c_{\pm}$ = concentration of activated complex.

In order to apply this theory to reactions in solution, certain assumptions must be made: (a) The approach of Eyring (41) is used, with the additional assumptions that the solutions are ideal and the activity coefficients are unity. (b) The time average collision frequency of reactants in solution is the same as in the gas phase. (c) The transmission factor is approximately unity.

Due to the inadequate knowledge of the properties of liquids, these assumptions cannot be rigorously evaluated. Therefore, only qualitative

interpretations can be made from the application of the theory of absolute reaction rates to the present investigation.

With recognition of the above assumptions, and assuming further that an equilibrium exists between the activated complex and the reactants, substitution of $\underline{K}^{\ddagger} = [\underline{B}^{\ddagger}]/[\underline{G}][\underline{H}^{+}]$ in (5) yields

$$\text{rate} = (\underline{kT}/\underline{h})\underline{K}^{\ddagger}[\underline{G}][\underline{H}^{+}] \qquad (6)$$

where $\underline{K}^{\ddagger}$ is the equilibrium constant for the formation reaction of the activated complex.

Combination of Equations (4) and (6) yields

$$\underline{k}_{obs.}/[\underline{H}^{+}] = (\underline{kT}/\underline{h})\underline{K}^{\ddagger}. \qquad (7)$$

By use of Equation (7) and fundamental thermodynamic relationships, the entropy of activation and free energy of activation may be calculated from the observed rate constants.

The free energy of activation may be calculated from the following equation:

$$\underline{k}_{obs.}/[\underline{H}^{+}] = (\underline{kT}/\underline{h}) \exp(-\Delta\underline{F}^{\ddagger}/\underline{RT}) \qquad (8)$$

which, when rearranged, is

$$\Delta\underline{F}^{\ddagger} = -2.303\ \underline{RT} \log(\underline{k}_{obs.}\ \underline{h}/[\underline{H}^{+}]\underline{kT}). \qquad (9)$$

Below is a sample calculation for the hydrolysis of methyl α-glucuronide at 80°C.

$\underline{k}_{obs.} = 6.886 \times 10^{-6}$ sec.$^{-1}$

$[\underline{H}^+] = 0.5$ molar

$\underline{h} = 6.62 \times 10^{-27}$ erg sec.

$\underline{k} = 1.38 \times 10^{-16}$ ergs per degree

$\underline{T} = 353°K.$

$\underline{R} = 1.987$

$$\Delta\underline{F}^{\ddagger} = (-2.303 \times 1.987 \times 353) \log \left[\frac{6.886 \times 10^{-6} \times 6.62 \times 10^{-27}}{0.5 \times 1.38 \times 10^{-16} \times 353}\right]$$

$\Delta\underline{F}^{\ddagger} = 28{,}630$ calories per mole

From (1), $\Delta\underline{H}^{\ddagger}$ was found to be 30,895 calories per mole. Utilizing

$$\Delta\underline{F}^{\ddagger} = \Delta\underline{H}^{\ddagger} - \underline{T}\Delta\underline{S}^{\ddagger}, \qquad (10)$$

$\Delta\underline{S}^{\ddagger} = 6.42$ e.u.